INVESTIGATING THE ROLE OF BIOCHEMICAL STRESS MARKERS IN ASSESSING DRIVING INDUCED FATIGUE

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CERTIFICATE

This is to certify that the work in the thesis entitled ***"Investing the role of biochemical stress marker in assessing driving induced fatigue"*** by ***Rakesh Buhlan*** in partial fulfilment of the requirements for the award of the degree of Master of Technology in Biotechnology Engineering in the department of Biotechnology and Medical Engineering, National Institute of Technology Rourkela is an authentic research work carried out by him under my supervision and guidance. To the best of my knowledge, the matter enclosed in this project work has not been submitted by any other university/institute for the award of any degree.

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It is said that curiosity is the mother of all inventions. If so, then I must be her favourite child. I dedicate this study to the tens and thousands of curiosity stricken science enthusiasts all over the world who work day in and out with a vision to understand the complexity of life and the living, to make the world a better place.

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Date: 01.06.2015 **RAKESH BUHLAN**

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ABSTRACT

Fatigue is a condition of extreme tiredness under the influence of which a person enters into a physiological state with reduced mental or physical performance capability. This can be results of physical and/or mental activities such as sleep loss or extended wakefulness, altered circadian phase, or workload. This state impairs a person's alertness and ability to safely operate a task or perform safety related duties. There are various groups of people which are considered to be at risk from the driver tiredness or fatigue. Among them are night workers, lorry drivers, people driving home after the night shift, company car drivers, etc. The main causes of fatigue are due to extended hours of monotonous work, absence of proper ventilation, stressful jobs, exposure to environmental factors like heat, noise, vibration, etc. Other major symptoms includes drowsiness, daydreaming, poor judgement, trouble in focussing, bad quality sleep, lack of sleep, and sleep demands induced by the internal biological clock, etc. In drivers prolonged driving increases the risk of getting fatigue, especially when they do not take sufficient rest/break in between driving. Under the influence of fatigue, there is continuous deterioration of driving performance, slow reaction time, reduced steering performance, poor concentration, unable to keep up distance between vehicles, and are susceptible to mentally withdrawn from the driving task. The main objective of this study was focused on assessing the severity fatigue with duration of simulated/real time driving from molecular level biomarkers i.e. CK-MB, lactate and platelet rich plasma serotonin. Briefly ten numbers each of non-trained and trained drivers were selected for the study. The non-trained drivers were subjected to simulated driving session over 12 hrs while the auto-rickshaw drivers were considered as trained drivers and the study was also carried out for 12 hrs. The proposed biomarkers were assessed from pooled blood samples in each group at three time points (0h, 6h and 12h). Further, the subjective feeling of fatigue was estimated using an array of subjective assessment tools (Epsworth Sleepness Scale, Berlin quaestionnaire and Beck's depression inventory). All the blood biomarkers showed significant trend across the time points with fatigue progression. Further, the parameters exhibited significant correlation with subjective assessment scores. The study concluded that the proposed blood biomarkers in combination might be used for fatigue staging in drivers reliably.

Key Words: Fatigue, sleep loss, drowsiness, simulated/real time driving, biomarkers, CK-MB, lactate and platelet rich plasma serotonin, subjective assessment tools

CHAPTER – 1

INTRODUCTION

1.1 FATIGUE

Fatigue can be define as a condition of extreme tiredness under the influence of which a person enters into a physiological state with reduced mental or physical performance capability. This can be results of physical and/or mental activities such as sleep loss or extended wakefulness, circadian phase, or workload. This state impair a person's alertness and ability to safely operate a task or perform safety related duties[1].

There are various groups of people which are considered to be at risk from the driver tiredness or fatigue. Among them are night workers, due to their body as not being acclimatised to the change in the sleeping pattern after first few days of the shift, lorry drivers, people driving home after the night shift, company care drivers, due to their unregulated driving hours, young drivers (18-25), as they have problem in admitting they are feeling tired and are more likely to push themselves to the limit just to avoid losing face, older drivers (50+), as they are more susceptible to falling sleep in the afternoon due to change in their body rhythms.

The main issues with the truck drivers which make them falls into the high risk groups and more susceptible to the fatigue are the shift works, long-hours continuously driving behind the wheel and regularly driving during the peak time of needed sleep. Along with these issues driving conditions and unable to maintain healthy life style are also major contributing factors to fatigue.

The main causes of fatigue are due to due to extended hours of uninteresting work, absence of proper ventilation, stressful jobs, exposure to environmental factors like heat, noise, vibration, etc[2]. Other major symptoms includes drowsiness, daydreaming, poor judgement, trouble in focussing, bad quality sleep, lack of sleep, and sleep demands induced by the internal biological clock, etc. in drivers prolonged driving increases the risk of getting fatigue, especially when they do not take sufficient rest/break in between driving

Under the influence of fatigue, there is continuous deterioration of driving performance, slow reaction time, reduced steering performance, poor concentration, unable to keep up distance between vehicles, and are susceptible to mentally withdrawn from the driving task. In order to compensate with the fatigue influences, drivers they may either increase the task demand such as driving faster than usual so that it can enhance adrenaline and attention level or they lower the task demand such as slowing down the speed or making larger following distances

to increase safety margins. But crashes and study of driving performances suggest that these strategies are not sufficient to overcome the risk involved[2].

The expanding number of transportation mishaps has turned into a significant issue for society. The car crashes will be generally diminished if discovering a judging standard to figure out if drivers stay alert or not, and make a notice to the drivers when they start to nod off, so it is important to research weariness location calculation which is likewise a key innovation in shrewd vehicles driving. The driver weariness issue has turned into an essential component for bringing about car crashes. Driver weariness is a real reason for auto mishaps, since lethargic drivers are not able to settle on fast choices, and they may have slower response times[3]. Therefore, numerous administrations have training system to alarm individuals to the perils of driving while tired, and drivers are urged to dodge conditions which may prompt driver weariness. Accordingly, how to direct and keep away from exhaustion and driving proficiently is one of the critical issues.

Fatigue has a very complex origin and is caused due to a combination of both central and peripheral factors. Hence, it has been neatly divided into Physical (or Peripheral) fatigue and Central Fatigue. Peripheral Fatigue (PF) occurs due to dysfunction of the muscles or impairment in neuromuscular transmission. It's caused after long hours of recreation, physical exertion, etc. Central Fatigue (CF) occurs due to specific alterations in the central nervous system and could originate because of stress, depression, boredom, sleep deprivation, etc. It basically results from impaired functioning of the central nervous system and even though it might not affect muscles directly, it reduces the capacity to work. The central fatigue starts at molecular level much before the symptoms occur. Of all the neurotransmitters responsible for CF, Serotonin or 5-HT (5-hydroxytryptamine) is the most studied one[4]. The Serotonergic system has been suggested as an important modulator of mood, emotion, sleep, appetite, and thus has been implicated in the control of numerous behavioural and physiological functions. 5-HT is unable to cross the Blood-Brain-Barrier (BBB), therefore cerebral neurons are required to synthesise it for themselves which increases the amount of Tryptophan (TRP). TRP is the precursor for synthesis of Serotonin, hence plays an important role by synthesis and release of 5-HT in the brain. This brain 5-HT is involved in control of tiredness, stimulation and mood, hence linked with central fatigue during sustained exercise. Meanwhile, Lactic acid is produced during chemical processes in the body when too little oxygen is present for usual processes (anaerobic metabolism). It serves as a major factor in muscle cramps and is produced in the tissues when conditions such

as heart attack or shock reduce the blood supply responsible for carrying oxygen. CK-MB is recognised as the leading cardiac serum marker and its determination has been proven to be more specific for myocardial necrosis. It is released after Myocardial Infarction and is detectable in plasma as early as 3-4 hours after the onset of symptoms.

Elevated CK-MB levels have been reported in significant percentages of patients with acute skeletal muscle trauma (59%), chronic muscle disease (78%) and chronic renal failure (3.8%). This is a notable shortcoming since its determination isn't tissue-specific. Any condition that causes muscle damage or interferes with muscle energy production can cause increase in CK levels[5].

Amid the starting piece of the trial, the example of progress for the FBI was comparable for both the control and restless arms of the study. Suddenly, both follows incorporated a huge increment in FBI (Fatigue Biomarker Index) qualities, recommending a critical reduction in exhaustion level, close midnight amid the move from day 1 to day 2. A comparable, however blunted, increment is seen in the control arm around the same time in the second night (day 2-day 3 move).

Of the salivary segments analysed till today, Melatonin has demonstrated most encouraging as an immediate measure of circadian beat. Levels of cortisol in serum and spit are known to be impacted by various figures expansion to circadian rhythms, including a mixed bag of intense and constant stressors, for example, a sleeping disorder, obstructive sleep apnoea, dejection and incessant weakness. Techniques to measure drowsiness have emerged from different behavioural, electrophysiological, hereditary, proteomic and metabolomics investigations of slumber and lack of sleep. Techniques for evaluating dozing risks have been proposed in light of changes in behavioural components, for example, reaction time. After a certain tranquil period, subjects finished the Profile of Mood States (POMS) review for appraisal of exhaustion level and StroopColor-Conflict Test (Stroop tests) for evaluation as subjective assessments[6]. While the Stroop tests are known to be affected by learning impacts, they were chosen for their simplicity of organization to gatherings of the size utilized as a part of this study.

Tiredness has more prominent impact on principle construct driving undertakings than in light of aptitude based errands, in spite of the fact that ability based assignments can't be utilized to give early markers of tired driving, deterioration of such errands may demonstrate the presence of other driving hindrances, for example, intoxication. Fatigue can be treated by taking proper rest because sleep restores the normal functionality of the nervous system.

And since it increases the risk of mishaps in roadways, aviation and military sector, it is essential to study the effects of sleep deprivation on performance, in order to develop methods to fight fatigue.

1.2 OBJECTIVES OF WORK

The overall aim of this research work was to measure the effects of fatigue on normal individuals after a prolonged session of driving and on professional auto-rickshaw drivers based on following objective:

1. To quantitatively determine the changes in the levels of blood biomarkers (CK-MB, Lactate and Serotonin) in young non-trained drivers (n=10) after a prolonged session of driving resulting in the fatigue using the respective biochemical assays.
2. To validate the changes in above objective parameters during fatigue progression using questionnaire based subjective assessment tools.
3. To compare the trend in blood biomarkers and subjective test scores with that of control condition (the same subjects without driving but resting for same time period) during fatigue progression using statistical analysis.
4. To analyse the effect of fatigue from real time driving on above biomarkers by quantifying them in pooled blood levels of auto-rickshaw drivers (n=10) before and after a day of driving and compare the results before and after the simulated driving in non-trained drivers.

CHAPTER – 2

LITERATURE REVIEW

2.1 INTRODUCTION TO FATIGUE

Fatigue can be define as a condition of extreme tiredness under the influence of which a person enters into a physiological state with reduced mental or physical performance capability. This can be results of physical and/or mental activities such as sleep loss or extended wakefulness, circadian phase, or workload. This state impair a person's alertness and ability to safely operate a task or perform safety related duties.

In general terms, the driver fatigue can be state as a condition when a driver experience the feeling of sleepiness, tired, exhausted or not having the energy to do anything, when driving a vehicle. Driver fatigue is a leading cause of fatal car crashes around the world. Researchers around the world have shown that tired drivers are a major road safety risk, both to themselves and to others. Across the Europe minimum 4000 people are killed each year as a result of driver tiredness[7]. In India, more than 40 thousand people die in the car crashes annually, in which about 30% of the total road fatalities is due to the driver fatigue only.

There are various groups of people which are considered to be at risk from the driver tiredness or fatigue. Among them are night workers, due to their body as not being acclimatised to the change in the sleeping pattern after first few days of the shift, lorry drivers, people driving home after the night shift, company care drivers, due to their unregulated driving hours, young drivers (18-25), as they have problem in admitting they are feeling tired and are more likely to push themselves to the limit just to avoid losing face, older drivers (50+), as they are more susceptible to falling sleep in the afternoon due to change in their body rhythms.

The main issues with the truck drivers which make them falls into the high risk groups and more susceptible to the fatigue are the shift works, long-hours continuously driving behind the wheel and regularly driving during the peak time of needed sleep. Along with these issues driving conditions and unable to maintain healthy life style are also major contributing factors to fatigue.

2.2 FATIGUE AND DRIVING ACCIDENTS

Over the past few years, there has been a lot of research done on occupational driver's health and work safety associated with driver's fatigue. It is one of the biggest problems of roadways. The impact of drivers fatigue isn't trivial and hence there have been a lot of studies done on it. One study showed that if a person is kept awake for a night, his performance

levels are as impaired as someone with high alcohol concentration in blood. There was a done by Harvard Medical School which suggested that 28% of adult drivers fell asleep during driving and 54% of them had feelings of drowsiness while they were handling the stick[1]. A study by Foret and Latin showed that train drivers feel sleepy during their work time due to their tight schedules. It deteriorated the quantity and quality of sleep which elevated their fatigue. Several other studies have advocated incidences of uncontrolled attacks of sleep during driving. There was a study that showed 50% of the road accidents were fatigue related on two of America's busiest roads. An Australian road safety organisation estimated that 30% disastrous crashes, 15% fatal accidents and 6% road mishaps were due to driver's fatigue. A World Health Organisation report of 2009 suggested that more people in India died due to road accidents than anywhere else in the world. So clearly, it's an important issue which raises serious questions regarding driver's fatigue and safety[7].

In different nations, studies show that male youthful drivers are more inclined to rest-related crashes because of their hectic schedules and propensity to drive high mileage on roads. It was also found that sleep related accidents were at peak in the early morning hours, i.e. from 2:00 Hours to 6:00 Hours. According to a study done by Horne, it was found that drivers were more prone to falling asleep at wheel at dusk than at 10 Hours. The probability of falling asleep on a monotonous road was also high in case of a long journey. New studies suggest that brain function is impaired at an equivalent level as it would be after staying awake for 17-19 hours. And the amount is equivalent to what 50-60 mL of alcohol would induce.

2.3 TYPES OF FATIGUE

Driving is a dynamic, complex activity which involves visual, cognitive and manual tasks. The driver has to form various strategic goals, monitor the vehicle system and the roadways environment, process information and make tactical action plans as well as execute control level activity.

Prolonged driving under stressful conditions leads to the driver fatigue. Depending on the nature of activity the fatigue may be physical or mental. Physical fatigue is a state when a person cannot continue functioning at their normal levels of physical ability, whereas mental fatigue is a psychological phenomenon where a person unable to concentrate properly on task at hand or is more slanted toward feeling sleepy[8].

2.3.1 PHYSICAL FATIGUE

It is a state under the influence of which a person's muscle cannot able to perform optimal physical performance i.e. activities and efficiency in terms of movements, power and coordination demanded by the body and is made more severe by continue intense physical exercise. Physical fatigue is influenced by the individual physical fitness and other factors such as sleep deprivation, excessive work load, repeated muscle movements, health, etc.

Driving requires many active physical activities such as handling movements of steering wheels, changing clutches and gears, regulating accelerator, break, use of horn, hand signals and indicator etc[9]. All these activities requires certain amount of energy to perform proper functioning because of which physical fatigue gradually increase with time and after a period of prolonged driving, the driver enters into the state of physically exhausted.

There are various factors which contribute to the physical fatigue are lack of muscle energy, decrease efficiency of neuromuscular junction and reduction in drive originating from the central nervous system (CNS).

2.3.2 MENTAL FATIGUE

It is a state under the influence of which a person is unable to perform an optimal cognitive and physical performance. It causes decrease concentration in task on hand and level of consciousness, feeling sleepy, impaired memory, etc. Mental fatigue often appeared together with physical fatigue, but not always and gradually increases with time due to prolonged activity.

Driving involves many active mental activities such as close attention, reasoning, memory, decision making, quick response, perception and recognition. All these mental activities involves synthesis of neurotransmitters which aid in the neural communication. The energy require to perform the proper communication is obtained by the consumption of the oxygen and glucose. The amount of glucose consumption by a particular region of the brain varies with the complexity of task or stimulus presented to the subject. Sensation of fatigue increases with the increased glucose consumption. Therefore increase in the mental activity demand due to prolonged work session, results into gradually increase in mental fatigue with time.

The driver fatigue can also be subcategorized based on the causal factors of fatigue into the Sleep-related (SR) and task related (TR) fatigue[10]. SR fatigue is affected by the time of

day, sleep-deprivation and extended hours of working, whereas TR fatigue is characterized by the task demand and duration.

2.3.3 SLEEP-RELATED (SR) FATIGUE

The factors contributing to the SR fatigue are Circadian rhythm, sleep-restriction and sleep-deprivation. Human is naturally adapted to sleep during night and awake during the daytime. This sleep/wake patterns follow the natural circadian rhythm of the body. An alertness dip in the early afternoon is also produced by the circadian rhythm, during which people are sleepier. The performance decrement are observed when there is troughs in the circadian rhythm. For instance, there are sleep related crashes occurs between 2 and 6:00 am and between 2 and 4:00 pm, which correspond to the troughs in the circadian rhythm.

There are other factors also which influence SR fatigue are homeostatic factors such as sleep-deprivation and duration of wakefulness[11]. The performance of an individual gets worse, longer they awake. Also insufficient/restriction sleep results into the increased sleepiness and declined work performance.

2.3.4 TASK-RELATED (TR) FATIGUE

The factors contributing to the TR fatigue are driving task and driving environment. TR fatigue can be active or passive in nature. Active fatigue is the most common form of TR fatigue. Active TR fatigue is the result of high demand driving condition such as poor visibility, high density traffic or completion of secondary tasks i.e. searching for an address, in addition to the driving. Passive fatigue is caused due to the underload driving conditions such as when roadways is monotonous and less traffic[12]. It is produced mainly when a driver is monitoring the driving environment for a prolonged time when most of the driving task is automated. This type of driving results in reduction in effort exerted on the task by the driver.

2.4 PHYSIOLOGICAL COMPONENTS OF FATIGUE

Physiological changes associated with fatigue which affects the body are changes in the brainwaves activity, head movement, eye movements, muscle tone and heart rate. It leads to the lowering in body temperature, blood pressure, respiration, heart rate and adrenalin production. Under the influence of it a person may undergo microsleep i.e. a brief nap that last for approximately four to five seconds.

Electroencephalogram (EEG) is the recorded electric potential from the exposed surface of the brain. EEG is one of the most valid indexes of alertness in person. It measures the different frequencies of brainwaves within brain. Therefore EEG provides the characteristic changes in signals during different types of mental and physical activities.

Driving involves activities like body movements, cognition, visual processing, decision making, etc. All these functions are associated with the certain change in the EEG signals.

The electrical activity of the brain is classified according to the rhythm. The frequencies of the brain waves varies from 0.5 to 100 Hz. These frequency bands are divided into four wave groups which includes, delta waves, theta waves, alpha waves and beta waves.

The delta waves frequency ranges from 0.5 to 4 Hz. These are recorded during the transition to drowsiness and sleep. Theta waves ranges from 4 to 7 Hz and these are recorded during the time of emotional stress like disappointment and frustration. Alpha waves ranges from 8 to 13 Hz and are usually recorded at relaxed state and are indicative of lack of visual processing and attention. Beta waves ranges from 13 to 13 Hz. These are recorded at the time of increase alertness, arousal and excitement. These are indicatives of intense mental stress.

2.5 PSYCOLOGICAL COMPONENTS OF FATIGUE

It relates to changes in the psychomotoric and cognitive function of the person. It also affects mood and motivation to work. These components are characterized by the feelings of exhaustion, discomfort, boredom, lack of motivation and interest to continue the task at hand.

This part of fatigue is also well known as mental fatigue and is associated with the reduced efficiency and alertness, unwillingness to work and impaired mental performance. This state leads to the decrease in reaction time, memory, vigilance, psychomotor coordination, information processing and decision making ability of the person[13].

2.6 CAUSES OF THE DRIVER FATIGUE

The principle known causes of driver can be divided into three main subcategory i.e. driver related, work related and environment related.

2.6.1 DRIVER RELATED

These includes circadian rhythm (biological clock), amount and quality of sleep, health conditions (physical and mental), number of waking hours, diet, fitness, home conditions, age, etc.

2.6.2 WORK RELATED

These includes length of work shift, time of day, corporate culture, lack of enough rest period, physical or mental workload, night work, etc.

2.6.3 ENVIRONMENTAL RELATED

These includes vehicle ergonomics, weather conditions, type of trip, monotonous of road, availability of rest areas, environmental stress (heat, noise, vibration), etc.

2.7 CIRCADIAN RHYTHM

The phenomenon under which human body is programmed to sleep at night and awake during daytime is known as circadian cycle or biological clock. It controls the body temperature, heart rate, blood-pressure, secretion of hormones, digestion and sleep cycle. This cycle is synchronised with 24 hour solar day and is regulated by the exposer to light and dark, which help it regulating the function of biological, physiological and metabolic activities. Therefore it has direct effect on mood, alertness, motivation and performance.

In case of drivers and shift workers, due to prolonged working and not having enough sleep these usual pattern and stable circadian cycle become desynchronised and body needs to adjust to being kept awake and alert when it naturally wants to sleep. It results into negative consequences such as release of melatonin, cortisol level and core body temperature. Melatonin is a hormone which is believed to induce sleep in humans[1]. This hormone is usually released in darkness is suppressed with night work and prolonged awaking, therefore drivers and shift workers experience more difficulty in sleeping during day time.

2.8 SLEEP

Sleep is state of natural rest in humans. It is an important factors for the humans to survive. The average person needs an uninterrupted 7-8 hours of sleep in every 24 hours on a daily basis. A good sleep prior to the driving is needed for proper driving with full alertness and in fully waking state without the having the need to sleep during driving. With quantity, there is also needed for the quality of sleep. If the normal sleep is regularly interrupted, this leads to the too little sleep which can lead to day-time fatigue. The factors which influence the quality of sleep among the others are sleep disorder such as sleep apnoea and narcolepsy, noisy and unpleasant sleeping environment[9]. The quality and quantity of sleep in a driver can be affected based on parameters such as amount of off duty time in between shifts and timetable, time of off-duty whether it is in morning, noon or night, resting environment, etc.

Sleep can be divided into two broad types based on the movement of the eye during sleep i.e. rapid eye movement (REM) and non-rapid eye movement (NREM) sleep. Sleep acts as a restorative function associated with physiological, neurological and psychological state. Sleep proceeds as a cycle of REM and NREM phases, which is approximately 90-110 minutes.

2.8.1 REM (RAPID EYE MOVEMENT)

In REM mode, the brain is active and body is in inactive state. This stage is characterized by cerebral activation, active motor inhibition and dreaming. In an electroencephalography (EEG) study, it's being characterized by low voltage and mixed frequency, which is similar to wakeful EEG state. REM improves the ability of an individual to sustain attention during waking hours and with intellectual functioning[10]. This state help in restoring our energy and consolidates memory.

2.8.2 NREM (NON-RAPID EYE MOVEMENT)

In NREM mode, the brain is in inactive state and body is in active state. There is very little dreaming in this state. It is encompasses of four stages i.e. stage 1 and stage 2 are considered to be 'light sleep' and stage 3 and stage 4 to be as 'deep sleep'. This state helps in enhancing the functions of immune system[11].

2.9 FATIGUE EFFECTS ON DRIVING

Fatigue can be said to be inversely proportional to the driving performance. Increase in fatigue results into the decrease driving performance with interval of time.

2.9.1 PARAMETERS INFLUENCED BY FATIGUE

The decrease in driving performance can be state in terms of following factors:

1. Increase reaction time
2. Decrease alertness
3. Impaired memory
4. Poor judgement and concentration
5. Reduced information processing state
6. Decreased field of vision
7. Increased drowsiness and sleepiness
8. Decrease motivation to carry out task
9. Restricted communication and interaction with surroundings
10. Increased irritability and aggressiveness towards others, etc.

2.9.2 DRIVING BEHAVIOUR DUE TO FATIGUE

The driver fatigue result due to the prolonged driving results into the withdrawn attention from the road and traffic requirements, leading to the impaired performance of the driver behind the wheel. Research studies have stated that a driver who is driving for more than 16 hours have driving skills comparable to the driver with 0.05 blood alcohol level. Whereas those who drives without going sleep for more than 24 hours are compared with driver with an illegal high BAC of 0, 1 g/l[12].

Several research studies have shown the fatigue influences on driving behaviour in specific ways:

1. Slower reaction time i.e. time taken to react to an sudden situation
2. Reduced information performance ability.
3. The accuracy of short term memories i.e. unable to memorize past few minutes of driving.
4. Reduced vigilance i.e. based on attention required tasks.

There are various warning signs behind the wheel which show that driver may be under the influence of fatigue:

1. Delayed breaking
2. Trouble in finding a comfortable position
3. Frequently yawning, nodding off
4. Difficulty in maintaining a constant speed.
5. Involuntary lane changes
6. No memory of last few kilometre of travelled
7. Failure to check mirror
8. Missing an exit
9. Hallucinations

2.10 MOLECULAR STUDY OF FATIGUE

The biochemical analysis of fatigue is done by the help three blood biomarkers i.e. CK-MB, Lactate and Serotonin.

2.10.1 CK-MB

Creatine kinase (CK) is a dimeric molecule which is composed of two subunits i.e. M and B, which are immunologically distinct from one another. It is mostly found in muscles, heart and brain. It consists of three different forms of CK-isoenzymes i.e. CK-MM, CK-MB and CK-BB. CK-MM is mostly found in the skeletal or voluntary muscles whereas CK-MB is mostly found in the muscles of heart. It is released into the blood only when there is damage to the heart muscles. The level of CK-MB increases significantly 4 to 6 hours, following myocardial infraction and usually peaks at 12 to 14 hours after the infract[14]. The levels return to normal after 24 to 48 hours, if there no further myocardial damage.

CK-MB assay test is done in order to detect and measure the level of CK-MB in the blood. The assay principle behind this is that CK-M fractions of CK-MM and CK-MB in the sample are completely inhibited by an anti-M antibody present in the reagent. Then the activity of CK-B is measured by the CK method. The final CK-MB activity is obtained by multiplying the CK-B activity by two.

2.10.2 LACTATE

Lactate is one of the product of cell metabolism and based on the body's pH it can either be present in the form of lactic acid or lactate. L-lactate is one of the important raw materials of glycogenesis produced by the active skeletal muscle and erythrocytes and is usually metabolised in the liver[14]. In normal person it usually present in lower concentration. Its concentration can be found in excess sometimes within the muscle cells, RBCs, brain and other tissues due to insufficient oxygen supply at cellular level or the primary way of producing energy in the body is disrupted. When tissue can't be supplied with sufficient oxygen to support aerobic oxidation of the pyruvate and NADH produces in glycolysis, NAD+ is regenerated from NADH by reduction of pyruvate to lactate. Increased concentration of lactate in the blood is thus an indicator of anaerobic metabolism i.e. blood flow to the tissues decrease and oxygen delivery is insufficient. In case of severe oxygen deprivation 'Lactic Acidosis' may occur.

L-lactate assay test was used to quantitatively determine the L-lactate concentration in the plasma, with the help of following reaction:

$$\text{L-Lactate} + O_2 \xrightarrow[\text{Lactate Oxidase}]{} \text{Pyruvate} + H_2O_2$$

$$H2O2 + \text{4-aminoantipyrine} + \text{TOOS} \longrightarrow \text{Purple product} + 4H_2O_2$$

TOOS-N-ethyl-N-(2 hydroxy-3-sulphopropyl)

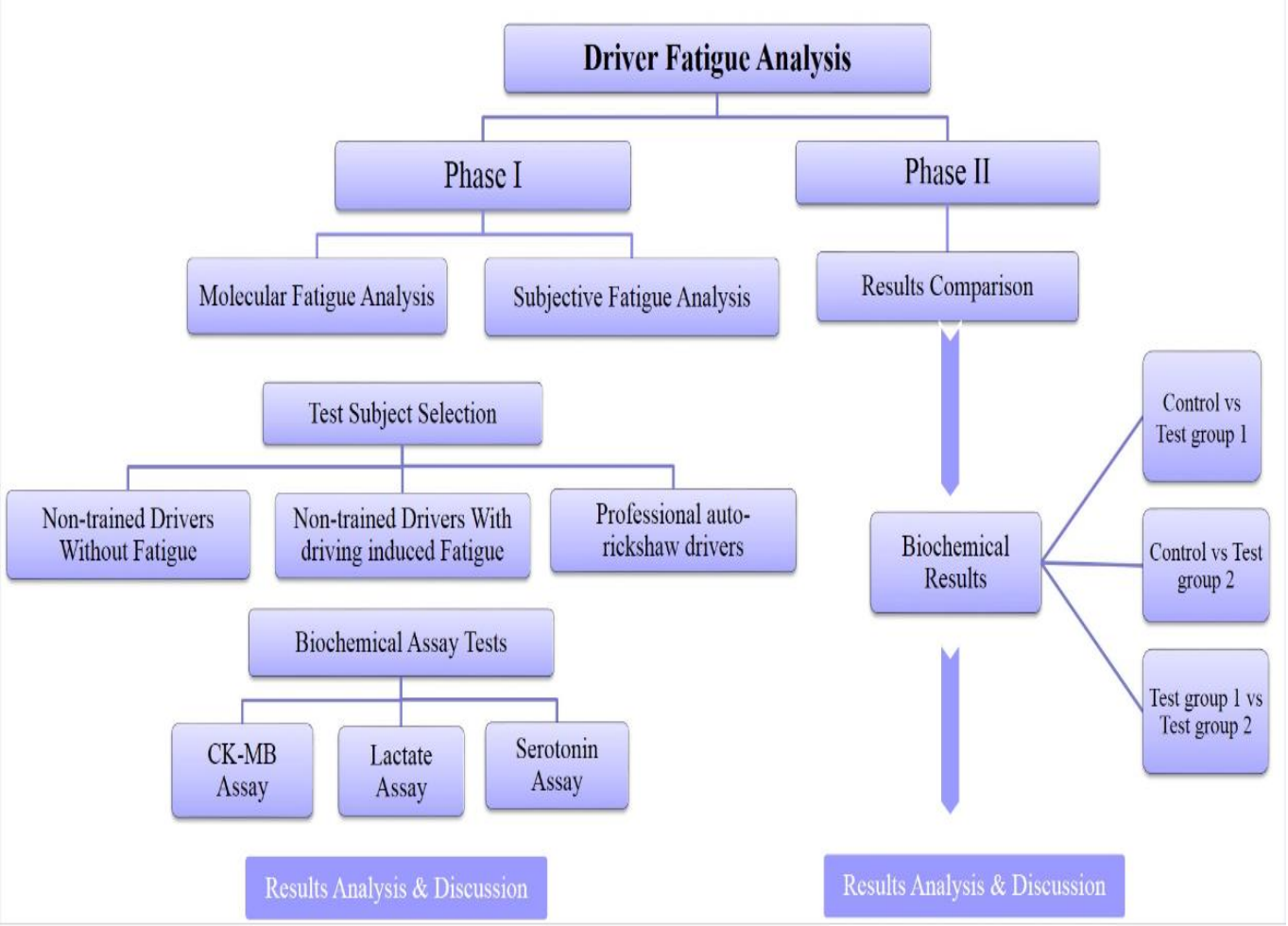
2.10.3 SEROTONIN AND CENTRAL FATIGUE

Fatigue has a very complex origin and is caused due to a combination of both central and peripheral factors. Hence, it has been neatly divided into Physical (or Peripheral) fatigue and Central Fatigue. Peripheral Fatigue (PF) occurs due to dysfunction of the muscles or impairment in neuromuscular transmission. It's caused after long hours of recreation, physical exertion, etc. Central Fatigue (CF) occurs due to specific alterations in the central nervous system and could originate because of stress, depression, boredom, sleep deprivation, etc. It basically results from impaired functioning of the central nervous system and even though it might not affect muscles directly, it reduces the capacity to work. The central fatigue starts at molecular level much before the symptoms occur[15]. Of all the neurotransmitters responsible for CF, Serotonin or 5-HT (5-hydroxytryptamine) is the most studied one. The Serotonergic system has been suggested as an important modulator of mood, emotion, sleep, appetite, and thus has been implicated in the control of numerous behavioural and physiological functions. 5-HT is unable to cross the Blood-Brain-Barrier (BBB), therefore cerebral neurons are required to synthesise it for themselves which increases the amount of Tryptophan (TRP). TRP is the precursor for synthesis of Serotonin, hence plays an important role by synthesis and release of 5-HT in the brain. This brain 5-HT is involved in control of tiredness, stimulation and mood, hence linked with central fatigue during sustained exercise.

CHAPTER – 3

PLAN OF WORK

3.1 BASIC RESEARCH PLAN OF WORK



CHAPTER – 4

MATERIALS AND METHODS

4.1 STIMULATED DRIVING SETUP

The stimulated driving setup consists of following devices

i. Personal Computer (Window 7, 4GB RAM, 1GB graphic card)
ii. Truck simulator software (Euro truck simulator 2)
iii. Driving force GT, Logitech, India (900-degree wheel rotation, Force feedback technology, Gas and brake pedals, Sequential stick shift)
iv. HD sound framework (Inspire M 4500 M 4500™, Creative)
v. A LCD projector
vi. One projector screen

All the devices were connected properly using standard protocols for electric device connections and the projector and the screen were set in such ways that, the driver can get a reasonable vision[13]. The entire setup was organized keeping drivers ergonomics in perspective.

4.2 TEST SUBJECTS SELECTION FOR PHASE I

For the Phase-I, the test subject selected were divided into the three groups. In the group 1, 10 normal individuals (male) of age between 20-30 years were selected. The proper consent form were signed by the participants for participating in the research. The objective and what were expected from them were clearly explained to them before admitting to the participation. These subjects acts as control group subjects for the research and were asked to give their blood samples in the morning after having a proper sleep for minimum 8 hours, so that they remain in normal healthy state without being affected by fatigue. These control groups were asked to come to the lab from 09:00 to 21:00 and allowed to go through normal day to day activity without undergoing any severe physical workload[1, 7]. The blood samples were collected from them at three different time intervals i.e. before, at 6 hour and after the 12 hour.

In the group II, 10 normal individuals (male) of age between 20-25 years were selected. These individuals were subjected to simulated driving for 12 hours in the simulated driving setup room. The blood samples were collected from them at three different time intervals i.e. before, at 6 hour and after the simulated driving session.

In the group III, the test subjected selected were the professional auto-rickshaw drivers. Total number of 10 auto-drivers was selected for the research purpose. The proper consent form

were signed by the participants for participating in the research. The objective and what were expected from them were clearly explained to them before admitting to the participation. The blood samples were collected from them at the time interval before at 6 hour and after of the normal driving day[8].

4.3 BLOOD SAMPLE COLLECTION

The blood sampling was done three times throughout the experiment from all the individuals. Briefly a tourniquet was wrapped around either arm (left or right arm altering at each time point) of the individual and was asked to flex the elbow as well as the fingers. From the anterior region of the elbow, 5ml of blood was collected by puncturing the brachial vein through 5ml disposable syringe fitted with 22G needles (BD Biosciences, India) after sterilizing it with a cotton swab soaked with 70% alcohol. 2ml of the blood was collected in a tube containing clot inhibitor (Na-EDTA) to obtain whole blood, and the remaining 2ml was collected in tubes containing clot activator(coated with Silicone gel, AkuSet™, Eastern Medikit Limited) to obtain serum, for the biochemical and the immunological assays[16]. The samples were vortexed 8 times for proper mixing followed by preservation in refrigerator at 2-8ºC. Later the samples were analysed for various blood biomarkers.

4.4 DESIGN OF EXPERIMENT

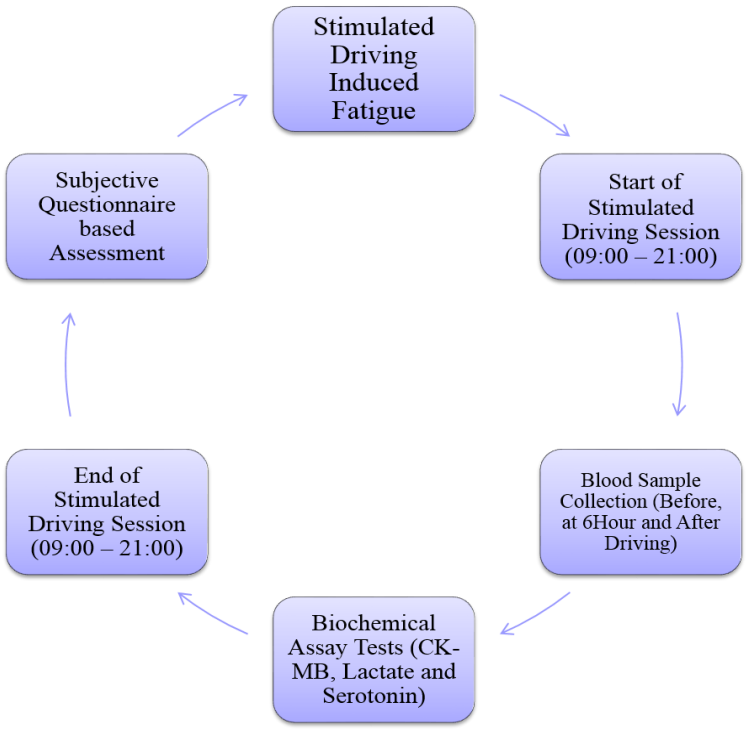


Figure 1 Experimental design for phase I study for driving induced fatigue determination.

4.5 BIOCHEMICAL ASSAYS FOR BLOOD BIOMARKER DETERMINATION

4.5.1 CK-MB ASSAY

Objective – For the determination of the CK-MB activity in the human serum[17].

Procedure – The CK-MB assay test was conducted based on the standard protocol available with Coral Clinical frameworks CK-MB assay kit. The biochemical analyser evolution 300 was used to determine the concentration of the CK-MB in plasma obtained from centrifugation of blood at 400Xg for 10 minutes at room temperature. The absorbance reading was recorded at 340nm at 37°C. The normal range of CK-MB found within the humans blood range between 0 to 24 U/L.

Pipette into clean dry test tube labelled as test (T)	
Addition sequence	T (37^o C)
L1	0.8mL
Sample	0.05mL
Incubate at assay temperature for 5mins	
L2	0.2mL
Mix well and read absorbance after every 1, 2 and 3mins	

4.5.2 LACTATE ASSAY

Objective – For the quantitative in vitro determination of L-Lactate in plasma[18].

Procedure – The lactate assay test was conducted based on the standard protocol available with the Randox research centres L-Lactate assay kit. The microplate reader was used to determine the absorbance at 595 nm in the blood plasma at 37°C. The normal range of lactate in blood plasma was range between 4.5 to 20 mg/dL.

	Reagent	Standard	Sample
Sample			10uL
Standard		10uL	
Reagent	1000uL	1000uL	1000uL
Incubate for 5mins at 37^oC			
Measure absorbance of A_{sample} and $A_{standard}$ within 30 minutes			

4.5.3 SEROTONIN ASSAY

Objective – For the quantitative in vitro determination of Serotonin in the blood[19].

Procedure - This assay test was conducted based on the standard protocol available with Labor Diagnostika Nord GmbH & Co. KG, serotonin ELISA assay kit. The absorbance reading of the sample (platelet rich plasma) was determine with microplate reader at 550 nm at 37°C. The normal range of serotonin within sample was in range 101 to 330 pg/ml.

Preparation of Standards			
Sample collection and storage			
Acylation (in acylation tubes)			
	Standards	Controls	Samples
Diluted Standard	20uL		
Diluted Controls		20uL	
Samples			20uL
Acylation buffer	25uL	25uL	25uL
Pipetting Scheme Research EIA			
	Standards	Controls	Samples
Standards	40uL		
Controls		40uL	
Samples			40uL
Serotonin Antiserum	50uL	50uL	50uL
Mix shortly and incubate for 15-20 hours at 2-8^{o} C; wash using wash-buffer concentrate			
Add Enzyme conjugate and incubate for 30mins at room temperature on a shaker			
Add substrate and incubate for 30mins at room temperature on a shaker			
Add stop solution and read absorbance at 450nm			

4.6 SUBJECTIVE ASSESMENT BASED FATIGUE ANALYSIS

The subjective assessments of fatigue takes into account a set of questionnaires which were asked to every subject towards the end of every step. For this appraisal, three standard conventions were taken. To be specific, Epworth sluggishness cycle that is a subjective measure of comprehending somebody's sleepiness. Beck Depression Inventory (BDI-II)[20], which comprises 21 multiple-choice self-reportable inquiries, have an essential aphorism to gauge the seriousness of depression and disinterest from the present undertaking in the subject; Berlin Questionnaire which is essentially used to focus the degree of torment and inability out of an intervention. All these evaluation instruments were slightly changed according to the necessity of the present study.

The inquiries were asked in a cooperative environment and the scoring was done on the consolidated perspective of the subject's self-assessment and the perception of one of the individuals from the examination group.

4.6.1 DESIGN

A survey study was design using the brief demographic questionnaire, Epworth Sleepiness Scale, the Berlin questionnaire and the Beck Depression Inventory. The participants selected for the study were non-trained drivers and auto-rickshaw drivers within the city. The final sample included 10 respondents.

4.6.2 INSTRUMENTATION

The overall questionnaire involved: A brief set of demographic and health related questions which included 8 items, 8 items were from the Epworth Sleepiness Scale (ESS), 10 items were from the Berlin questionnaire (BQ) and the Beck Depression Inventory (BDI) consisted of 20 items.

The ESS was used to measure a person’s level of daytime sleepiness with 8 item questionnaire. It measures a person’s probability to doze off or fall sleep in 8 different daily situations which is scored on a 4 point scale (0-3). The final score is a summation of 8 different categories with a final score within the range of 0-24. A high score is indicative of a greater amount of daytime sleepiness[21].

The BQ was used to determine the risk of having sleep apnoea with 10 items within 3 categories. The three categories correspondence to presence and frequency of snoring, wake

time sleepiness and fatigue, and hypertension. Those participants who scored consistently positive for two or more categories are considered to be at high risk for sleep apnoea.

The BDI questionnaire is used to determine the severity of depression within the participants. The scoring scale is from 0-3 for 21 items. The items from 1-13 refers to psychological symptoms, and from 14-21 refers to physical symptoms. These are used to determine mood, pessimism, sense of failure, guilt, self-dissatisfaction, punishment, self-accusation, self-dislike, crying, suicidal ideas, irritability, fatigue, social withdrawal, appetite, etc. The BDI ranges from a score of 0-63, a high scale is representative of severe depression.

4.6.3 PROCEDURE

All the participants were given the explanatory statement regarding the research study and respective consent form was also implied through completion of the questionnaire. The total time interval of approximately 20 minutes were taken for the completion of questionnaire.

4.6.4 DEMOGRAPHICS

The participants in this project work were between the ages of 20-30. All the participants in the study were Male and were tested healthy. Subsequently, a record of the demographics has been maintained. The assessments used were quite simple and understandable, so all the returned forms could be studied easily.

4.6.5 EPWORTH SLEEPINESS SCALE (ESS)

It is a self-directed test. It is generally utilized as a part of the field of sleep-medicine as a subjective measure of understanding someone's drowsiness[22]. The test comprises of eight circumstances in which you rate your propensity to wind up drowsy on a size of 0, no shot of snoozing, to 3, high risk of napping. When you complete the test, sum up the assigned values of your reactions. Your aggregate score is in view of a size of 0 to 24. The scale gauges whether you are encountering excessive tiredness that commands any medical help.

4.6.6 BERLIN QUESTIONNAIRE

The Berlin Questionnaire is a basic sleep apnoea screening poll used to rapidly identify the danger (low to high) of sleep disturbed breathing,[23] i.e. it is utilized to recognize the danger of sleeping issues and the disorders. The questionnaire comprises of 3 classes and the danger is in light of the reactions to individual questions and general scores in the symptom categories.

4.6.7 BECK DEPRESSION INVENTORY

Beck Depression Inventory is a set of questionnaire used for the evaluation of depression level in patients, experts and so on. The latest form (BDI-II) is a nonexclusive instrument that can be utilized on various age groups over thirteen, and is made out of polls that identify with melancholy level (sadness, irritation and so on.) , perceptions (guilty feeling, self-dislike, feelings of punishment and so forth) and additionally, if any physical side effects[24].

CHAPTER – 5

RESULTS AND DISCUSSION

5.1 MOLECULAR ANALYSIS OF FATIGUE IN NON-TRAINED DRIVERS WITHOUT STIMUATED DRVING SESSION

5.1.1 CK-MB LEVEL IN BLOOD

The individuals from this group acts as a control group for the research study. The average CK-MB level in the blood found to be at approximately 0.163 U/L at three different time zone i.e at 0hr, 6hr and 12hr. These levels are within the normal range found in the human body. These results indicates that with increase in daytime, there was no increase in CK-MB level which implement that with normal activities within the whole there was no significant effect on CK-MB level[25]. So it can be concluded from these results that there was no significant effect on heart muscles from day to activity and with proper sleep and resting condition.

5.1.2 LACTATE LEVEL IN BLOOD

The average lactate level in blood found in this control group within the 12hr interval from 09:00 to 21:00 at three different time interval i.e. 0hr, 6hr and 12hr are 14.14, 14.08 and 14.15 mg/dL respectively. These levels were found to be within the normal range of lactate within the body. These results indicate that with increase in daytime, there was no increase in lactate level which implement that with normal activities within the whole there was no significant effect on lactate level. So it can be concluded from these results that there was no significant effect on aerobic energy production metabolism and hence there was no conditions found which can cause insufficient oxygen supply within blood from day to activity and with proper sleep and resting condition[26].

5.1.3 SEROTONIN LEVEL IN BLOOD

The average serotonin level in blood found in this control group within the 12hr interval from 09:00 to 21:00 at three different time interval i.e. 0hr, 6hr and 12hr are 280.58, 252.46 and 254.02 pg/ml respectively. These results indicate that with increase in daytime, there was no increase in serotonin level which implement that with normal activities within the whole there was no significant effect on serotonin level[27]. So it can be concluded from these results that there was no significant effect on mood in the subjects during the study time and hence there was no conditions found which can lead to depression from day to activity and with proper sleep and resting condition.

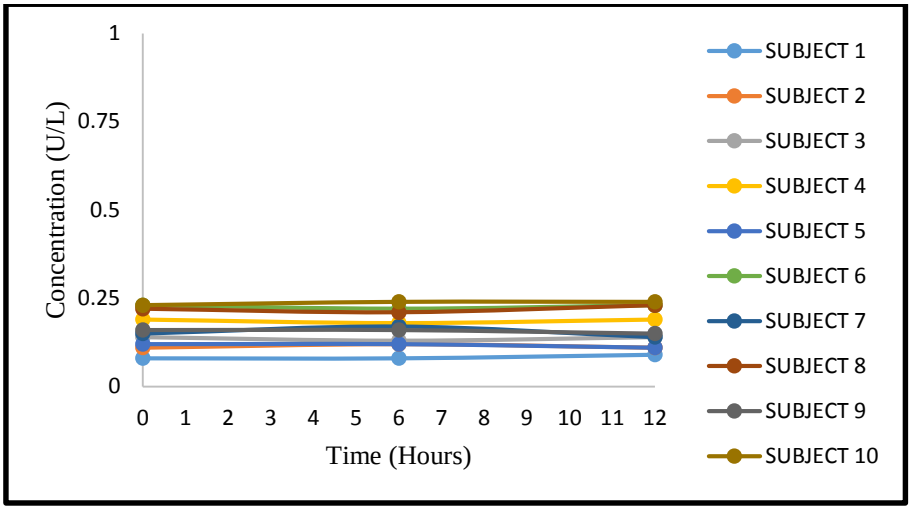


Figure 2 CK-MB levels in blood of Control group of 10 test subjects.

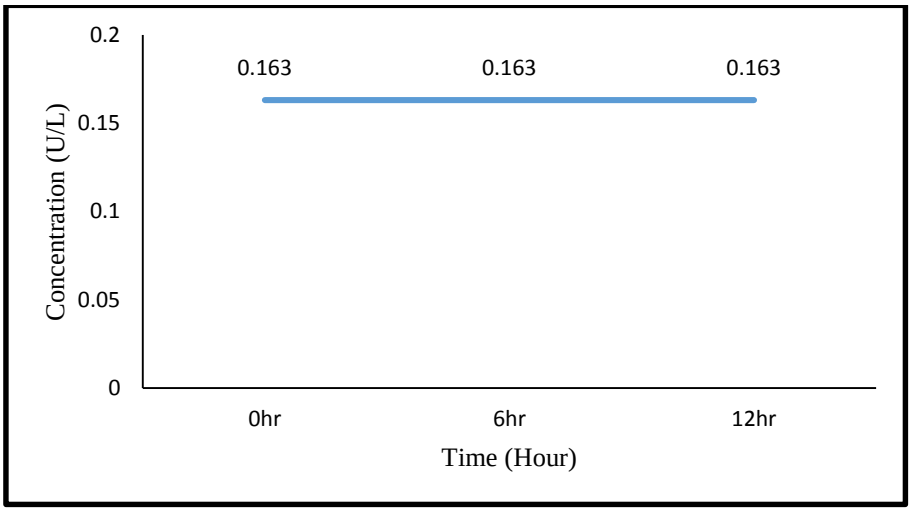


Figure 3 Mean CK-MB levels in blood of Control group of 10 test subjects.

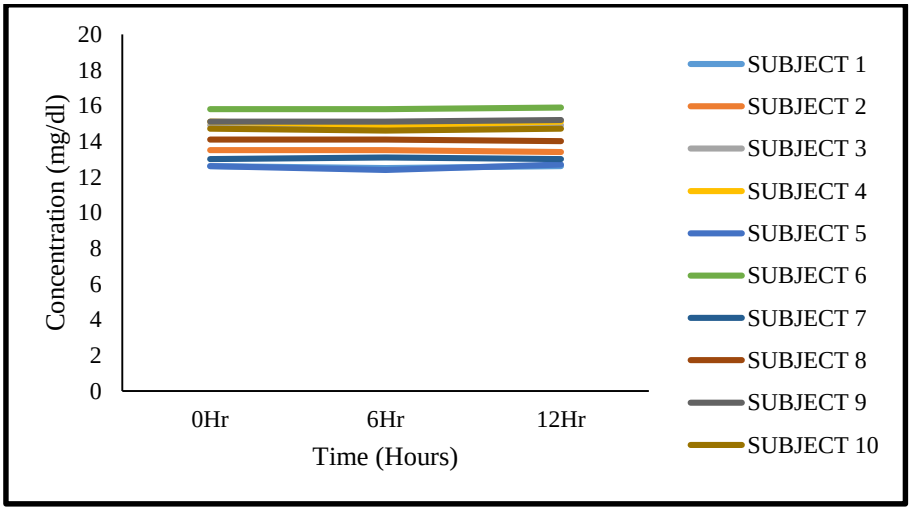


Figure 4 Lactate level in blood of Control group of 10 test subjects.

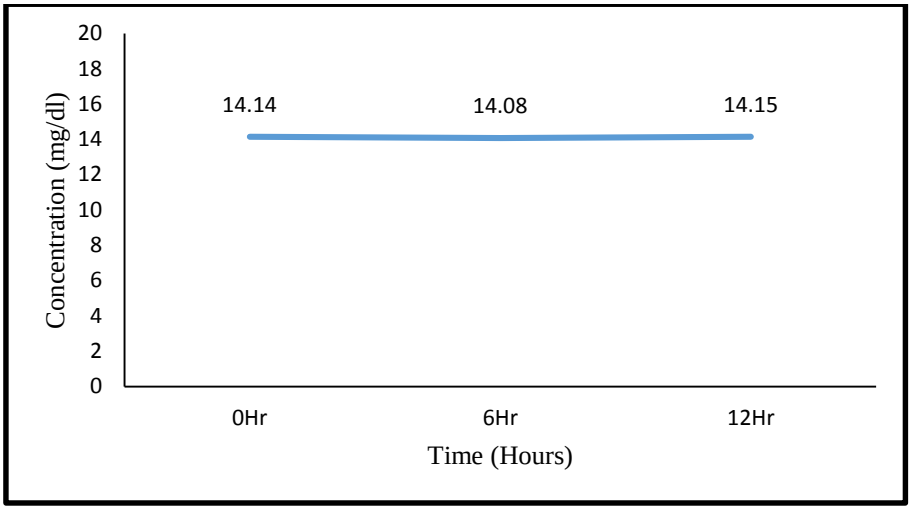


Figure 5 Mean Lactate level in blood of Control group of 10 test subjects.

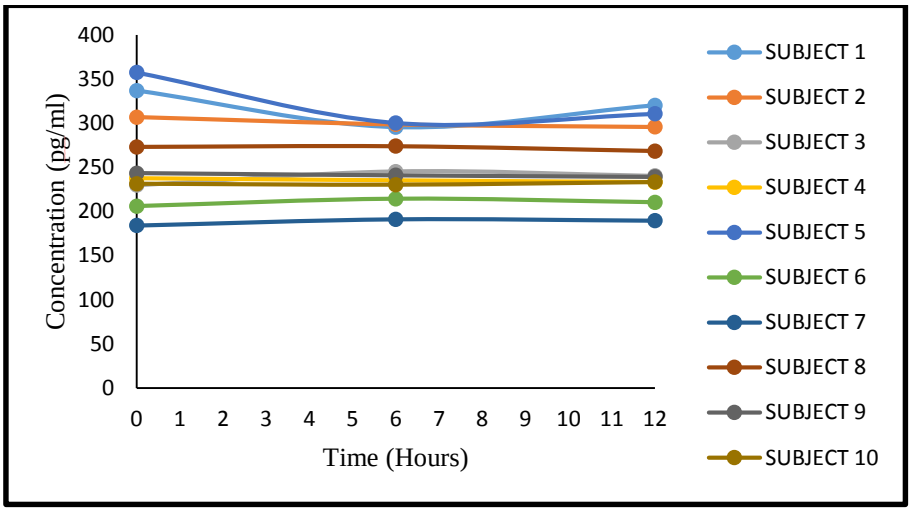


Figure 6 Serotonin level in blood of Control group of 10 test subjects.

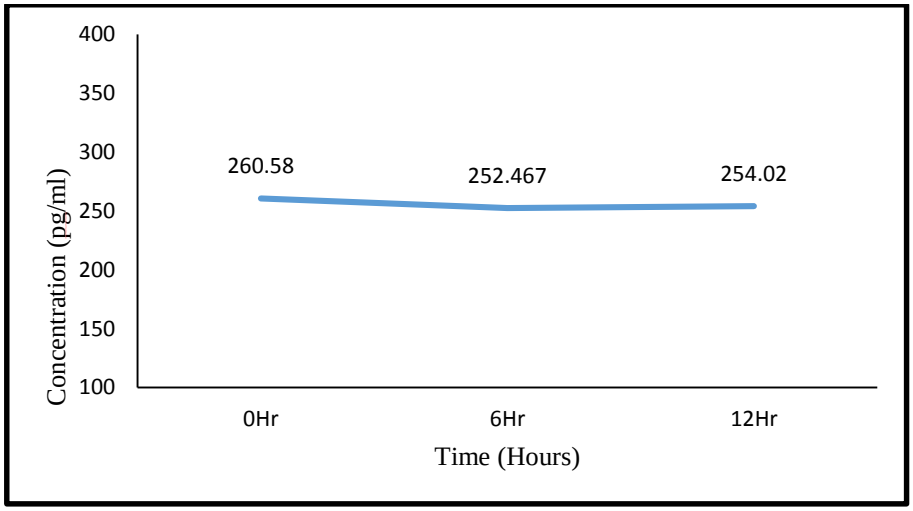


Figure 7 Mean Serotonin level in blood of Control group of 10 test subjects

5.2 MOLECULAR ANALYSIS OF FATIGUE IN NON-TRAINED DRIVERS WITH STIMUATED DRIVING INDUCED FATIGUE

5.2.1 CK-MB LEVEL IN BLOOD

The individuals from this group acts as a Test group-I for the research study. The average CK-MB level in the blood found to be at approximately 0.221, 0.474 & 1.701 U/L at three different time zone i.e at 0hr, 6hr and 12hr after the start of stimulated driving for 12hrs (09:00-21:00). The results indicate that the CK-MB level increases with increase in time of stimulated driving. Though the levels are within the normal range for humans but results shows that it kept on rising with time and driving induced fatigue in test subjects. These results conclude that with increased in stimulated driving fatigue, CK-MB level rises but not to the level where it can effects the heart muscles[28].

5.2.2 LACTATE LEVEL IN BLOOD

The average lactate level in blood found in this Test group-I within the 12hr interval from 09:00 to 21:00 at three different time interval i.e. 0hr, 6hr and 12hr are 36.62, 55.23 and 83.68 mg/dL respectively. The results indicate that the lactate level increases with increase in time of stimulated driving. Before starting of stimulated driving lactate level were in normal range but with increase in driving induced fatigue their level had crossed the normal range and was found thrice the time of average level at 0hr. This indicate that with driving induced fatigue in drivers it caused their metabolism to adapt to the secondary energy production pathway i.e[29]. Anaerobic which resulted into decomposition of lactate within the body. This causes insufficient supply of oxygen within the body.

5.2.3 SEROTONIN LEVEL IN BLOOD

The average serotonin level in blood found in this Test group-I within the 12hr interval from 09:00 to 21:00 at three different time interval i.e. 0hr, 6hr and 12hr are 211.07, 61.43 and 7.37 pg/ml respectively. The results indicate that serotonin level decreases with increase in time of stimulated driving. Before the start of stimulated driving their levels were within the normal range which was also indicative of their happy mood. But with increase in driving induced fatigue their levels fall below the normal range and after 12hr of driving it was found to be very least, which was also indicative of their off-mood state[30].

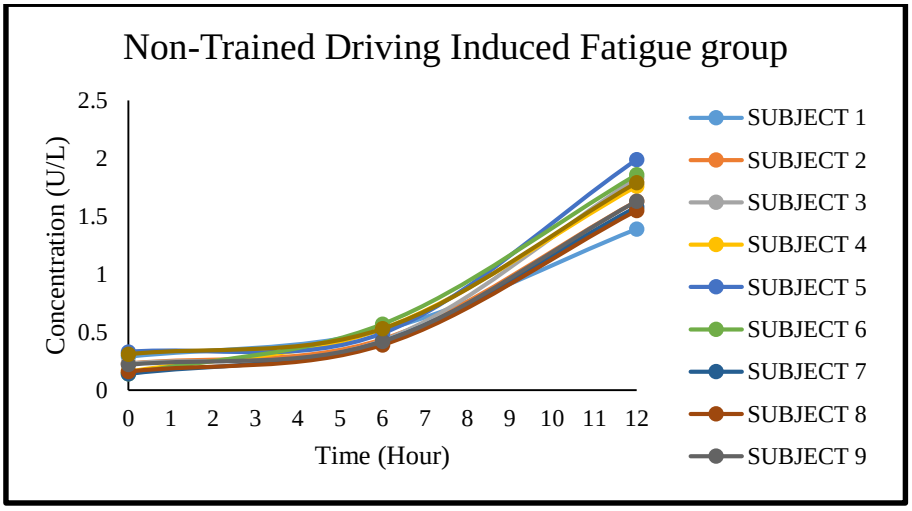


Figure 8 CK-MB levels in blood of Test group-I of 10 test subjects.

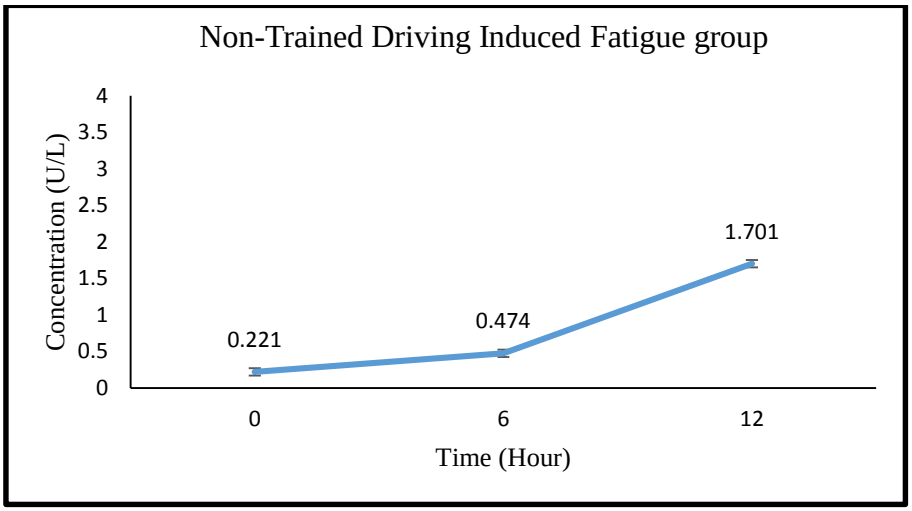


Figure 9 Mean CK-MB levels in blood of Test group-I of 10 test subjects.

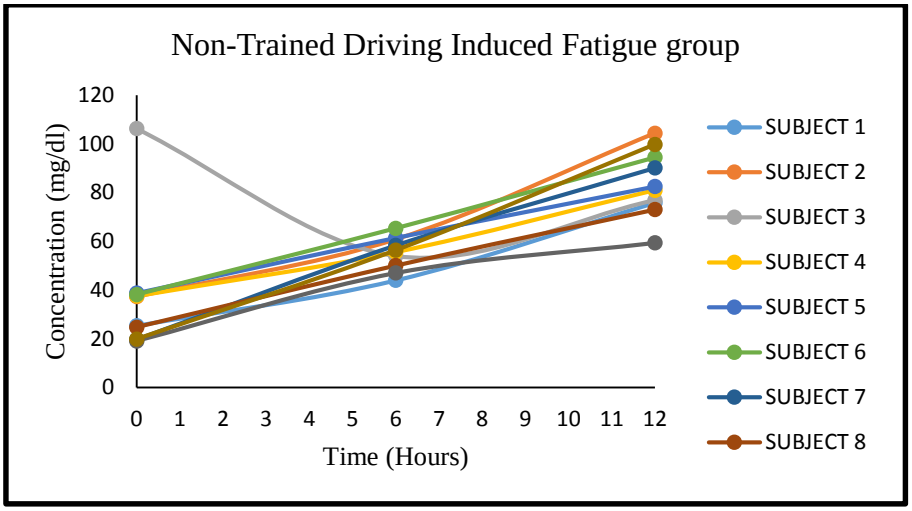


Figure 10 Lactate levels in blood of Test group-I of 10 test subjects.

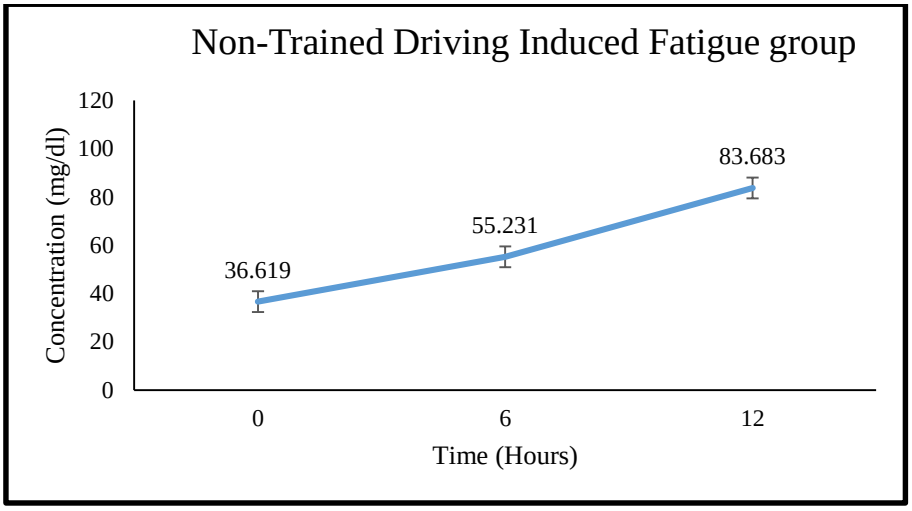


Figure 11 Mean Lactate levels in blood of Test group-I of 10 test subjects.

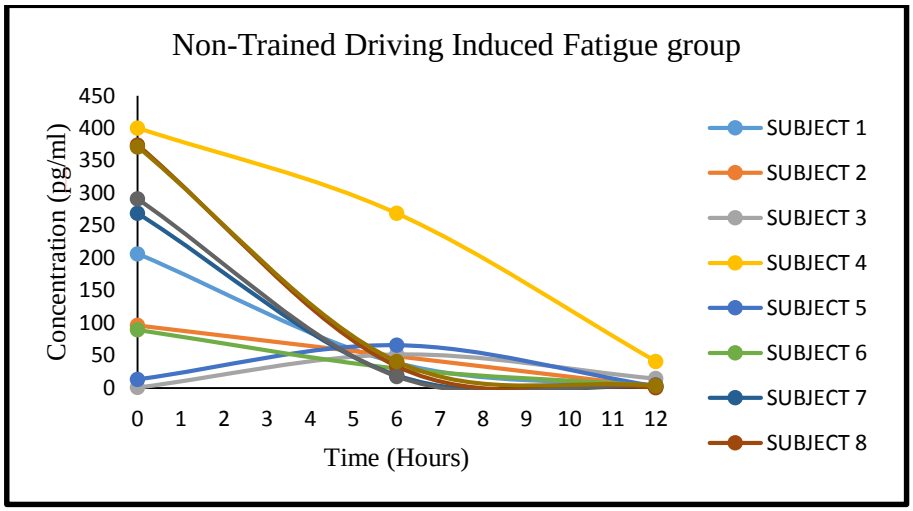


Figure 12 Serotonin levels in blood of Test group-I of 10 test subjects.

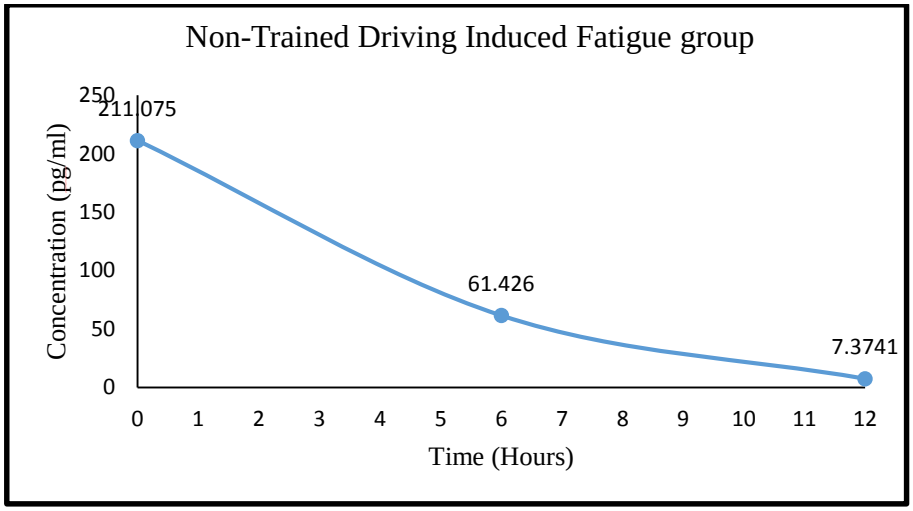


Figure 13 Mean Serotonin levels in blood of Test group-I of 10 test subjects.

5.3 MOLECULAR ANALYSIS OF FATIGUE IN TRAINED AUTO-RICKSHAW DRIVERS BEFORE AND AFTER DAY OF DRIVING

5.3.1 CK-MB LEVEL IN BLOOD

The individuals from this group acts as a Test group-I for the research study. The average CK-MB level in the blood found to be at approximately 3.041, 3.758 & 4.397 U/L at three different time zone i.e at 0hr, 6hr and 12hr after the start of stimulated driving for 12hrs (09:00-21:00). The results indicate that the CK-MB level increases with increase in time of driving. Though before and after driving the levels are within the normal range for humans but results shows that it kept on rising with time with driving induced fatigue in test subjects. These results conclude that with increased in stimulated driving fatigue, CK-MB level rises but not to the level where it can effects the heart muscles[31].

5.3.2 LACTATE LEVEL IN BLOOD

The average lactate level in blood found in this Test group-I within the 12hr interval from 09:00 to 21:00 at three different time interval i.e. 0hr, 6hr and 12hr are 29.28, 46.83 and 57.29 mg/dL respectively. The results indicate that the lactate level increases with increase in time of stimulated driving. Before starting of stimulated driving lactate level were in normal range but with increase in driving induced fatigue their level had crossed the normal range and was found thrice the time of average level at 0hr. This indicate that with driving induced fatigue in drivers it caused their metabolism to adapt to the secondary energy production pathway i.e. Anaerobic which resulted into decomposition of lactate within the body. This causes insufficient supply of oxygen within the body.

5.3.3 SEROTONIN LEVEL IN BLOOD

The average serotonin level in blood found in this Test group-I within the 12hr interval from 09:00 to 21:00 at three different time interval i.e. 0hr, 6hr and 12hr are 211.07, 61.43 and 7.37 pg/ml respectively. The results indicate that serotonin level decreases with increase in time of stimulated driving. Before the start of stimulated driving their levels were within the normal range which was also indicative of their happy mood. But with increase in driving induced fatigue their levels fall below the normal range and after 12hr of driving it was found to be very least, which was also indicative of their off-mood state.

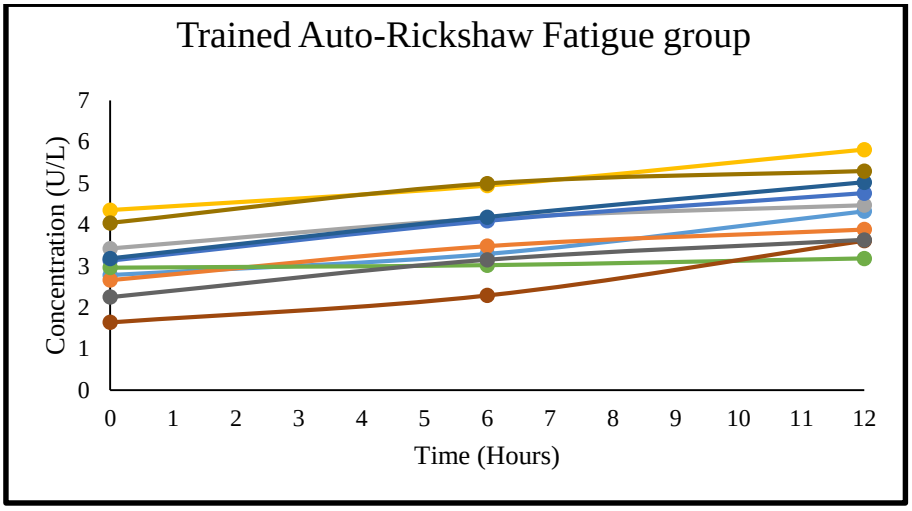


Figure 14 CK-MB levels in blood of Test group-II of 10 test subjects.

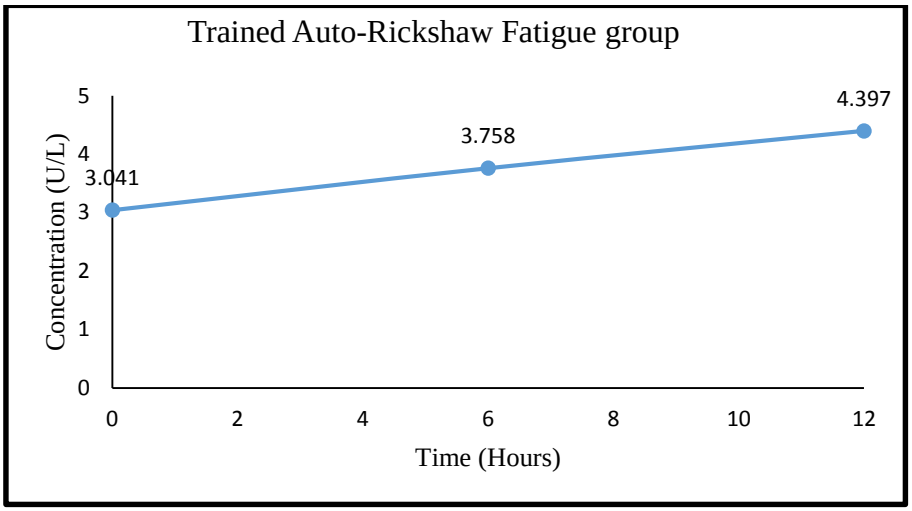


Figure 15 Mean CK-MB levels in blood of Test group-II of 10 test subjects.

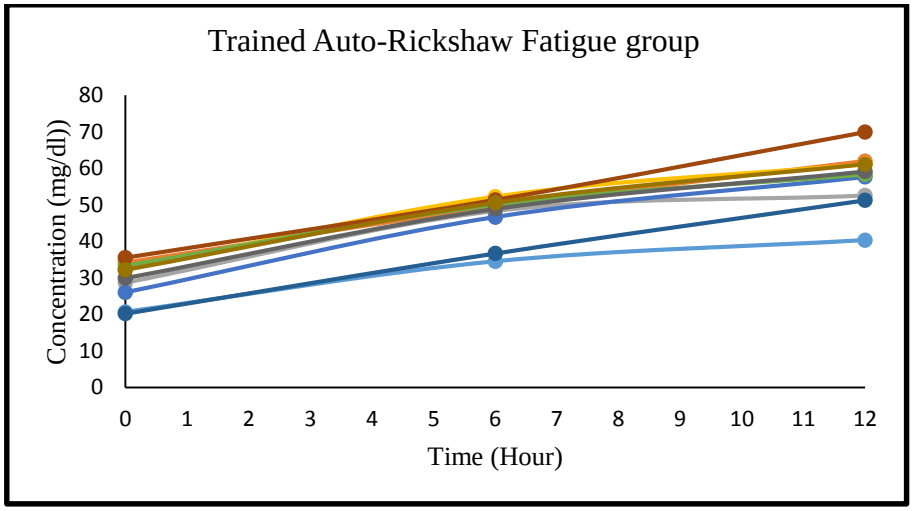


Figure 16 Lactate levels in blood of Test group-II of 10 test subjects.

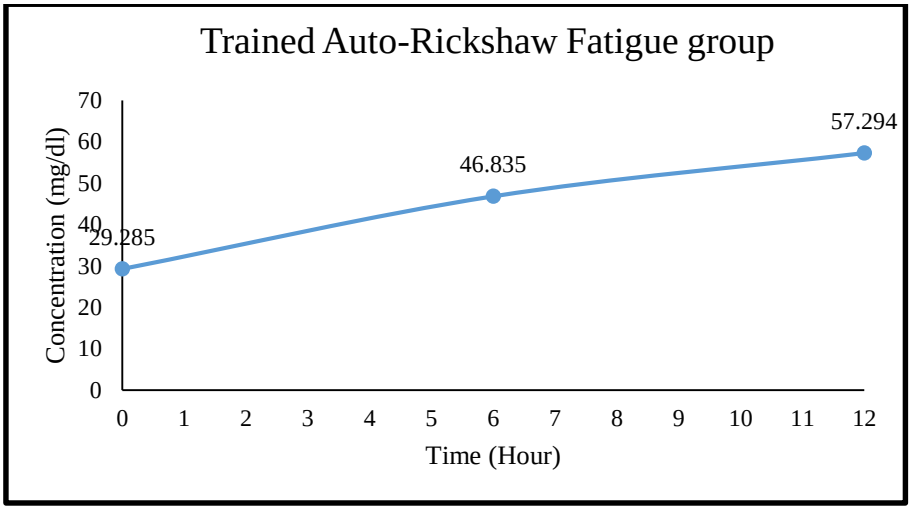


Figure 17 Mean Lactate levels in blood of Test group-II of 10 test subjects.

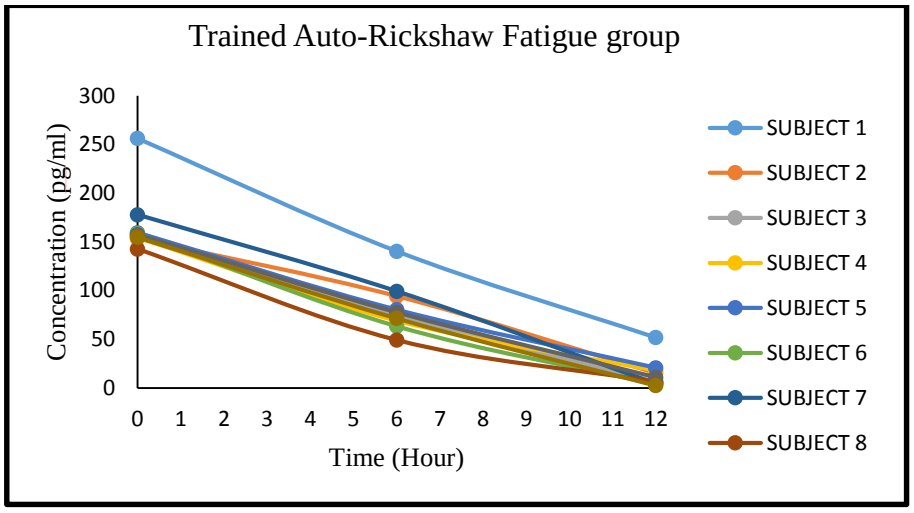


Figure 18 Serotonin levels in blood of Test group-I of 10 test subjects.

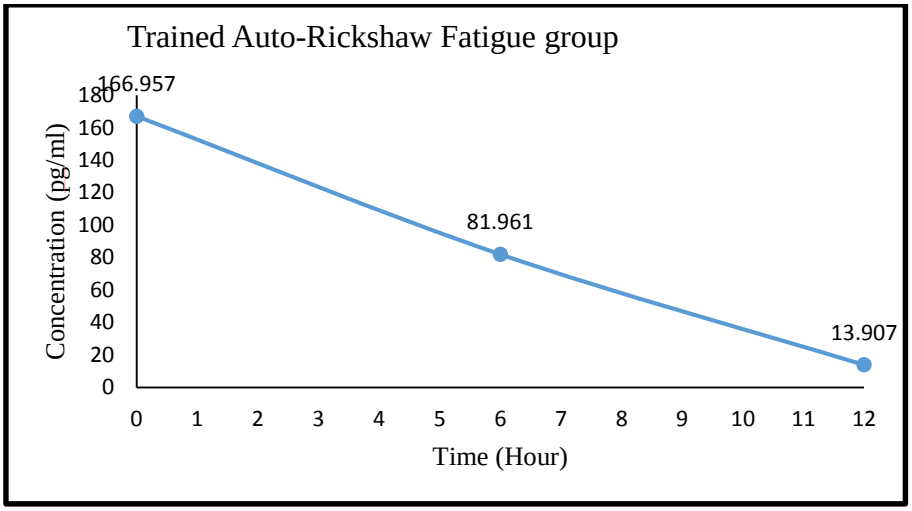


Figure 19 Mean Serotonin levels in blood of Test group-II of 10 test subjects.

5.4 SUBJECTIVE QUESTIONNAIRE BASED ANALYSIS OF FATIGUE IN NON-TRAINNED DRIVERS WITH STIMULATED DRIVING INDUCED FATIGUE

5.4.1 DEMOGRAPHICS STUDY RESULTS

The test subjects of the study were between the age groups of 21 to 30; 6 of them being of 21 to 25 age whereas the other 4 being of the 26-30 age group. All of them were male. The experience of the majority differed as 60% of the subjects had below 1 year of experience and 40% had an experience between 1 to 5 years. 80% of the subjects had experienced fatigue after the stimulated driving session of 12hrs and all of them admitted to having their fatigue affect their performance at work. None of them had been diagnosed with narcolepsy, restless leg syndrome and central sleep apnoea, prior to this experiment[32]. The number of people who admitted that fatigue had affected their work-performance was 8 at the end of driving session.

5.4.2 EPWORTH SLEEPINESS SCALE (ESS) RESULTS

Under the given different scenario to determine the dozing off or sleepiness scale in test subjects 1, it was found that maximum number individual will go to sleep if they allow to go to lying down to rest at noon with score of 0.6 after entering into the induced fatigue state. But before the start of driving session and up to 6hrs of driving none of them reported to be this much dizziness or feeling sleepy[32].

5.4.3 BERLIN QUESIONNAIRE RESULTS

This test was done to determine whether the tests individuals were under the effect of sleep apnoea or not. But with the survey results 100% of the test subjected reports that they were not having any sleep related issues as none of them opted for the snoring.

5.4.4 BECK DEPRESSION INVENTORY

This test was done to determine whether the tests individuals were under the effect of depression or not. Within the scoring of the test score, it was estimated that none of the test subjects were facing issues regarding depression[33].

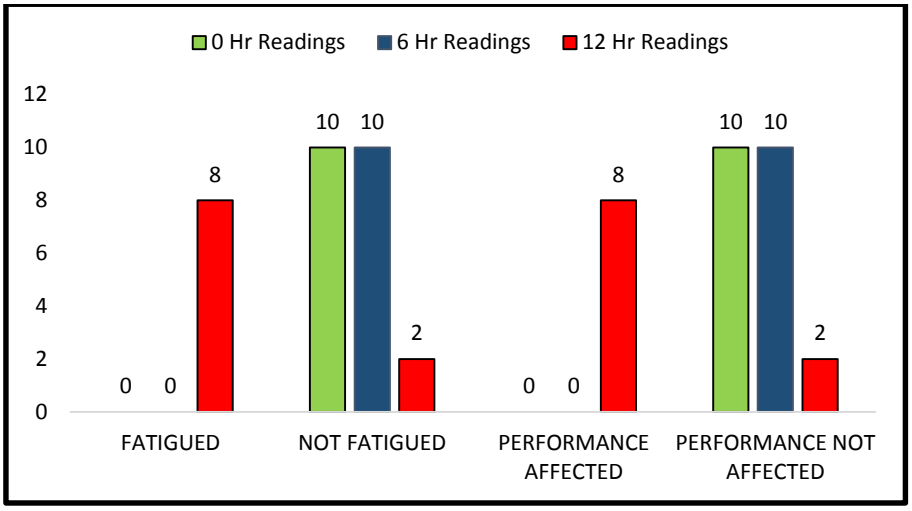


Figure 20 Demographics Results of driving induced fatigue group

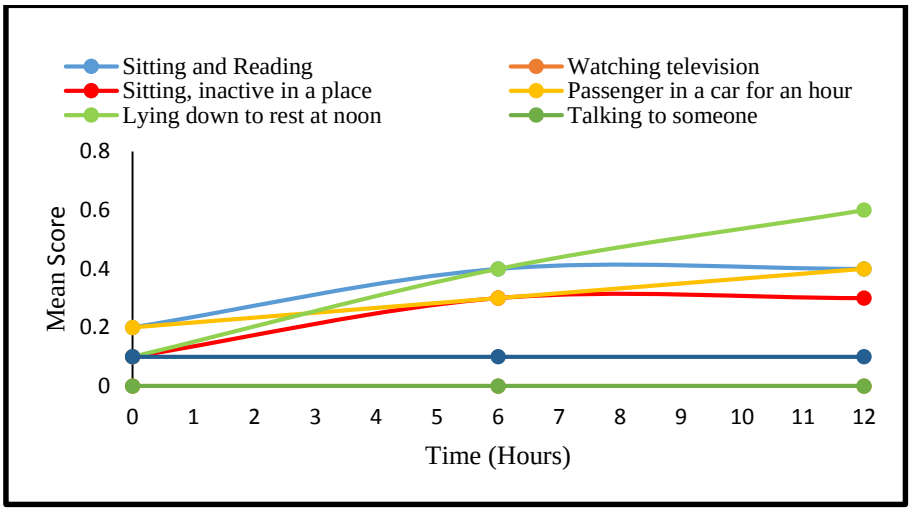


Figure 21 Epworth Sleepiness Scale Results of driving induced fatigue group of 10 subjects.

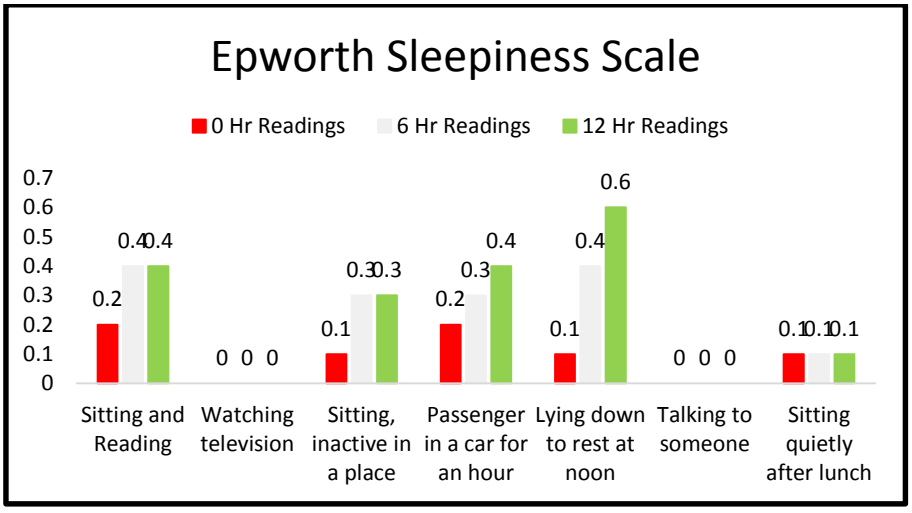


Figure 22 Epworth Sleepiness Scale Results of driving induced fatigue group at different time.

5.5 SUBJECTIVE QUESTIONNAIRE BASED ANALYSIS OF FATIGUE IN TRAINED AUTO-RICKSHAW DRIVERS WITH DRIVING INDUCED FATIGUE

5.5.1 DEMOGRAPHICS STUDY RESULTS

The test subjects of the study were between the age groups of 21 to 30; 4 of them being of 21 to 25 age whereas the other 6 being of the 26-30 age group. All of them were male. The experience of the majority differed as 40% of the subjects had below 1 year of experience and 60% had an experience between 1 to 5 years. 80% of the subjects had experienced fatigue in the past 6 month and all of them admitted to having their fatigue affect their performance at work. Six of them had been diagnosed with narcolepsy, restless leg syndrome and central sleep apnoea, but none of them had taken the medicine against it. The number of people who admitted that fatigue had affected their work-performance was 8 at the end of driving session.

5.5.2 EPWORTH SLEEPINESS SCALE (ESS) RESULTS

Under the given different scenario to determine the dozing off or sleepiness scale in test subjects II, it was found that maximum number individual will go to sleep if they allow to go to lying down to rest at noon with score of 2.9 after entering into the induced fatigue state. Beside this, second highest score was recorded in sitting as a passenger in car for an hour and sitting inactive in a place.[33] The subjects felt dangerously sleepy which could pose undesirable dangers for them, their partners and the normal road users. Significant improvement was seen while ‘someone was sitting and talking to them’, rather than the drivers driving alone for long hours. It was also seen the risks of accidents were high if the person had had alcohol in lunch or dinner.

5.5.3 BERLIN QUESIONNAIRE RESULTS

This test was done to determine whether the tests individuals were under the effect of sleep apnoea or not. It was recorded that 40% of the study subjects were under high risk of sleep apnoea and 30% were under low risk. The mean values for people who snore and their frequency of snoring being the highest at 0.76 and 0.64 with standard deviations of 0.4830 and 0.4538 respectively. Now, the BQ suggested that around 4 out of the 10 test subjects were at high-risk of sleep apnoea, 3 were at low-risk and nothing could be said about the rest 3, since they were unaware of some of their health problems and had varied answers[34]. Sleep apnoea could lead to memory loss, depression, stroke, etc. It is disturbing to find that so many individuals were at high risks and were completely unaware of it. It was also noted that snoring is a habit amongst many

subjects and that their snoring bothers other people, hence it could lead to disturb in their partners' sleep pattern too. None of the

Subjects nodded off or fell asleep during the driving period, so that was a relief. Though, some admitted to have had signs of moderate dozing during the lunch hours. People were also asked if they had suffered from high blood pressure and 30% of them could not give us an affirmation. Proper diagnosis was suggested to those at high risk.

5.5.4 BECK DEPRESSION INVENTORY

It is a scale which tests how depressed a person is. This was very important to the study to record the change in mood and depression levels of the test subjects, during simulated driving. This would give us a bigger and clearer picture of why the biochemical parameters are affected. Most of the subjects were recorded to have mild depression at the start of the study, i.e. comprised as sample study. The results showed that 10% of the subjects had mild depression, 40% had moderate depression while the rest 50% had severe depression, towards the end of the study[34]. According to Beck Inventory, depression has two constituents namely, Affective component which consists of eight items i.e. mood/pessimism, feelings of past failure, guilty feeling, punishment-like feeling, self-hatred, self-criticalness, worthlessness, suicidal feelings; whereas the Somatic component consists of loss of pleasure, sadness, agitation, loss of zeal and energy, sobbing, loss of interest, prickliness, change of appetite, vagueness, tiredness, concentration difficulties, change in sleep pattern and loss of interest.

The BDI questionnaire showed that 50% of the test subjects were under severe depression while 40% were under moderate depression. As the day started, their depression levels were recorded to be minimal or mild. But with the advance of the strenuous activity, their depression levels showed major changes. It was noted that once the person is depressed, the most affected criteria is one's sleeping pattern. They find it difficult to cry, even if they want to. It makes them irritated for most of the time and hence, they find it difficult to concentrate on their work. Most of them admitted that their depression state affects their quality of work and it leads to withdrawal from the job undertaken. They become indecisive, due to which they find it difficult to make any snap judgement or take reflex actions on time, once faced with a calamity or accident. These traits are highly undesirable in drivers and needs to be looked out for.

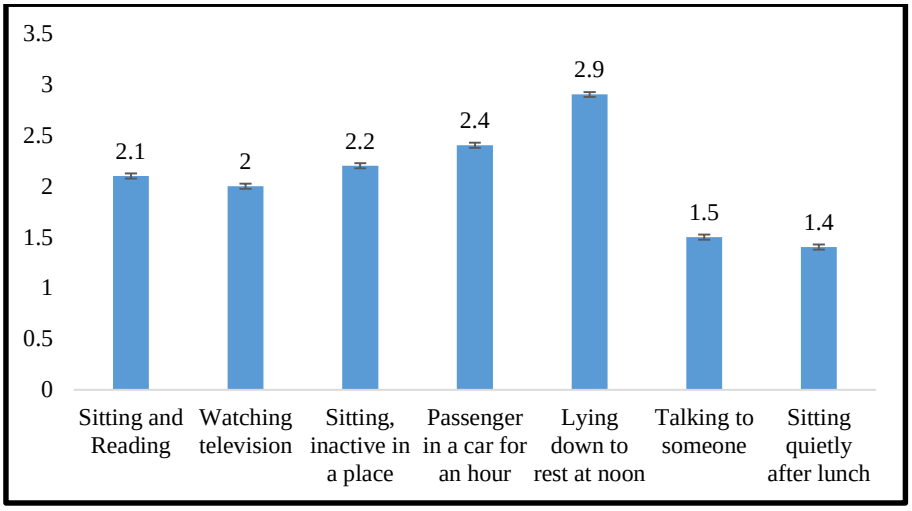


Figure 23 Epworth Sleepiness Scale Results of driving induced fatigue group of 10 subjects.

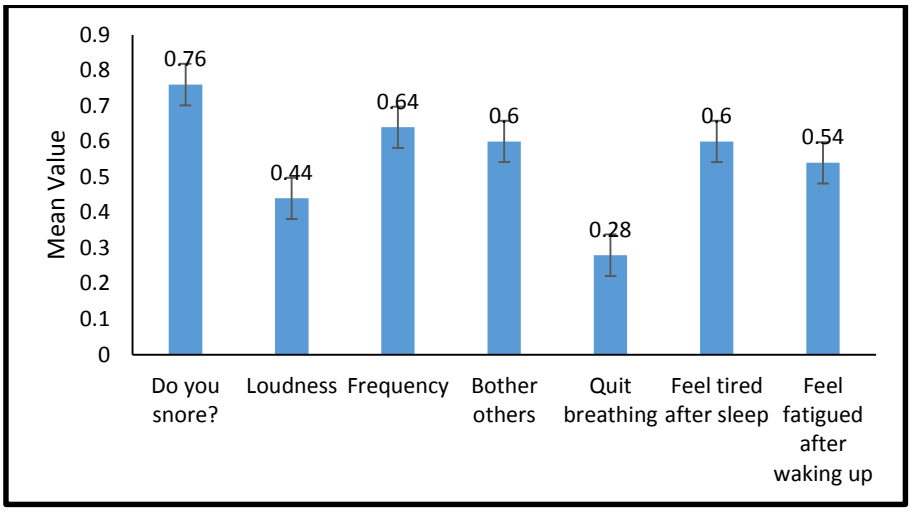


Figure 24 Berlin Questionnaire Results of driving induced fatigue group of 10 subjects.

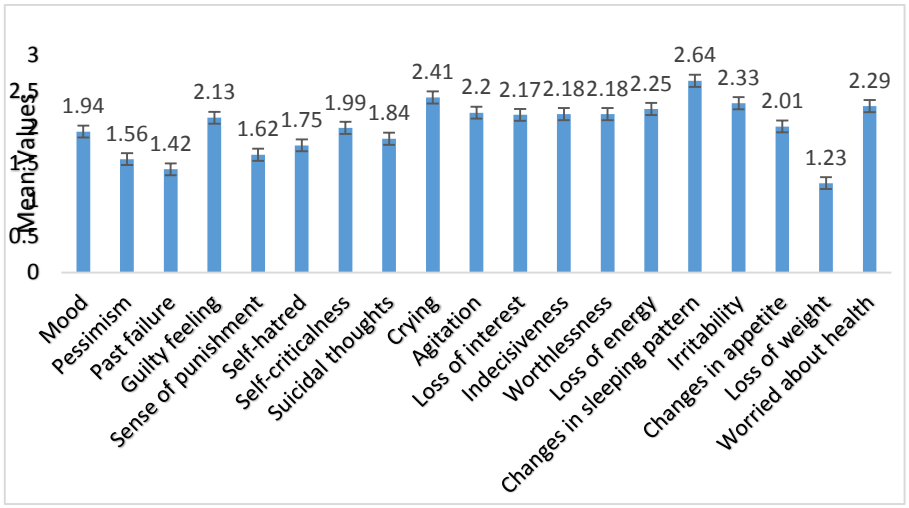


Figure 25 BDI Results of driving induced fatigue group of 10 subjects.

5.6 INTER COMPARISON OF MOLECULAR ANALYSIS RESULTS IN CONTROL GROUP Vs TEST GROUP I

5.6.1 CK-MB LEVEL IN BLOOD

An independent-samples t-test was conducted to compare whether or not driver fatigue is associated with the increase in CK-MB level in blood plasma within the driver. For this the results of the control group was compared with the non-trained driving induced fatigued group. So after the stimulated driving session of 12hrs, the mean value of CK-MB in blood of test group-I was found to be 1.70±0.055 and to that of control group was 0.16±0.017. The results obtained from these was found to be extremely statistically significant with p value 0.387. This state that with increase in induced driving fatigue, the CK-MB level in blood of non-trained drivers with stimulated driving also increased. Though it was found that the increased CK-MB level was well within the normal range of the human but the results shows that CK-MB can be used as a biomarker for determination of progression of fatigue within an individual with prolonged work time.

5.6.2 LACTATE LEVEL IN BLOOD

An independent-samples t-test was conducted to compare whether or not driver fatigue is associated with the increase in lactate level in blood plasma within the driver. For this the results of the control group was compared with the non-trained driving induced fatigued group. So after the stimulated driving session of 12hrs, the mean value of lactate in blood of test group-I was found to be 83.68±4.30 and to that of control group was 14.15±0.370[35]. The results obtained from these was found to be extremely statistically significant with p value 0.099. This state that with increase in induced driving fatigue, the CK-MB level in blood of non-trained drivers with stimulated driving also increased. The increased in lactate level crossed the normal value after 6hrs of driving and was recorded atleast four times that of before driving session.

5.6.3 SEROTONIN LEVEL IN BLOOD

An independent-samples t-test was conducted to compare whether or not driver fatigue is associated with the increase in serotonin level in blood plasma within the driver. So after the stimulated driving session of 12hrs, the mean value of lactate in blood of test group-I was found to be 7.37±3.923 and to that of control group was 254.02±13.691. The results obtained from these was found to be extremely statistically significant with p value 0.306[35].

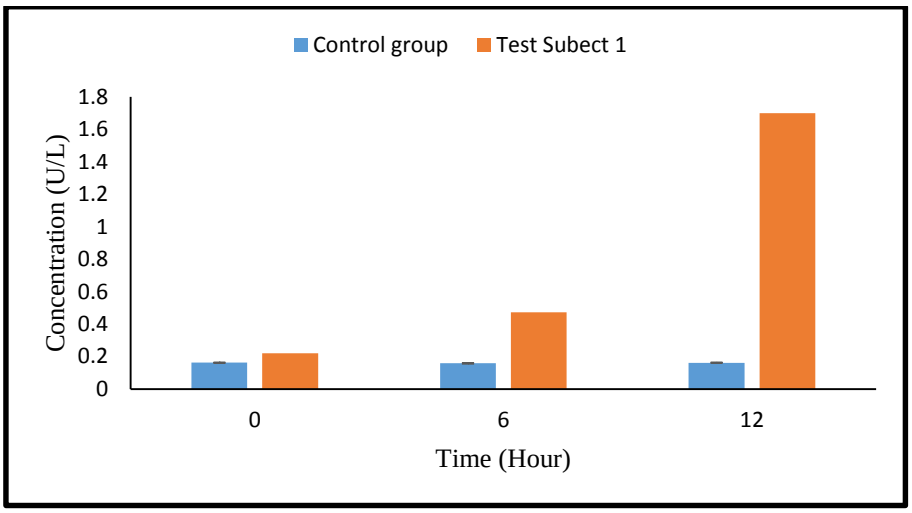


Figure 26 Bar graph Comparison of mean CK-MB level between control and test subject group 1

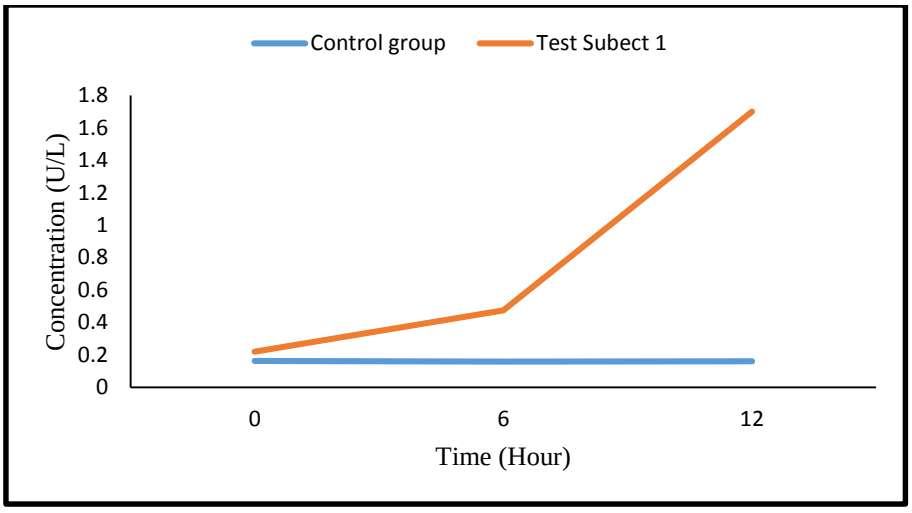


Figure 27 Timeline Comparison of mean CK-MB level between control and test subject group 1

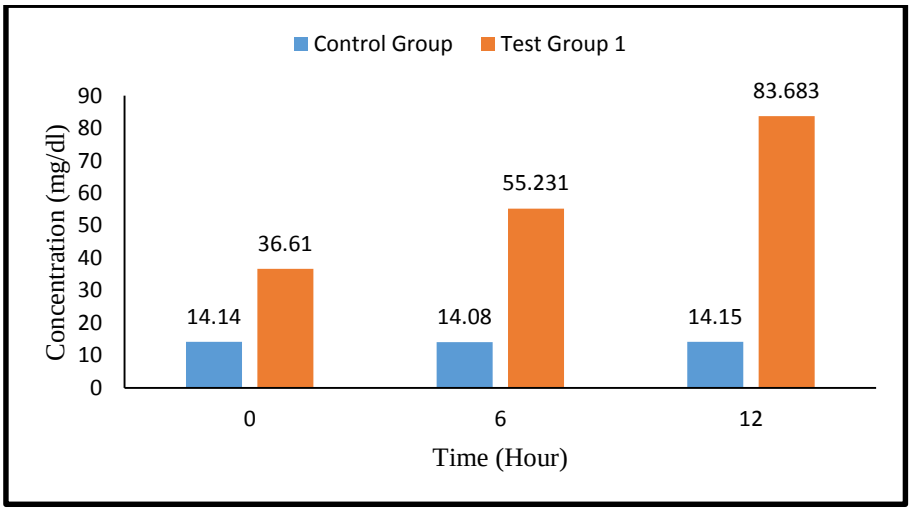


Figure 28 Bar graph Comparison of mean lactate level between control and test subject group 1

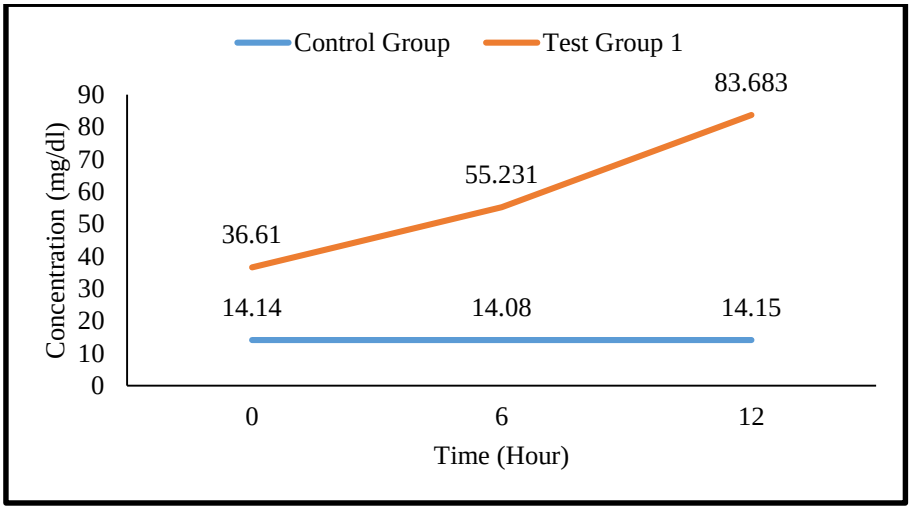


Figure 29 Timeline Comparison of mean lactate level between control and test subject group 1

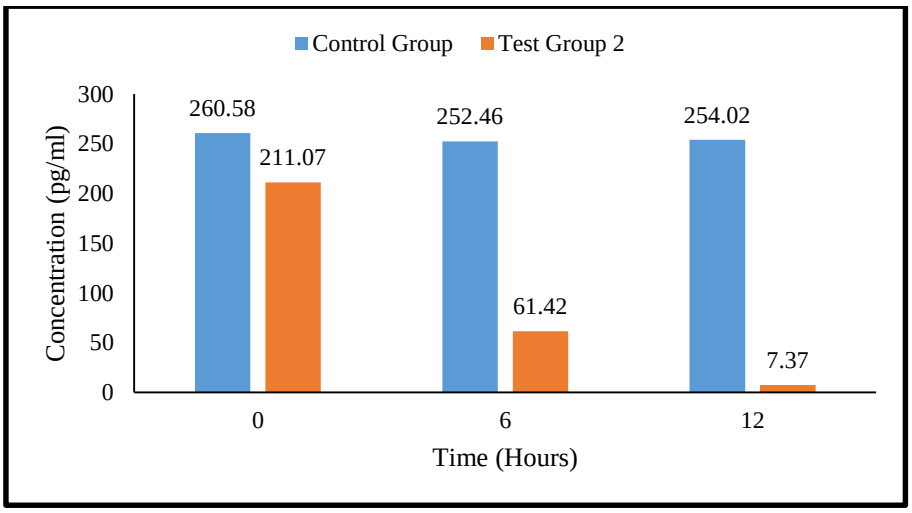


Figure 30 Bar graph Comparison of mean serotonin level between control and test subject group 1

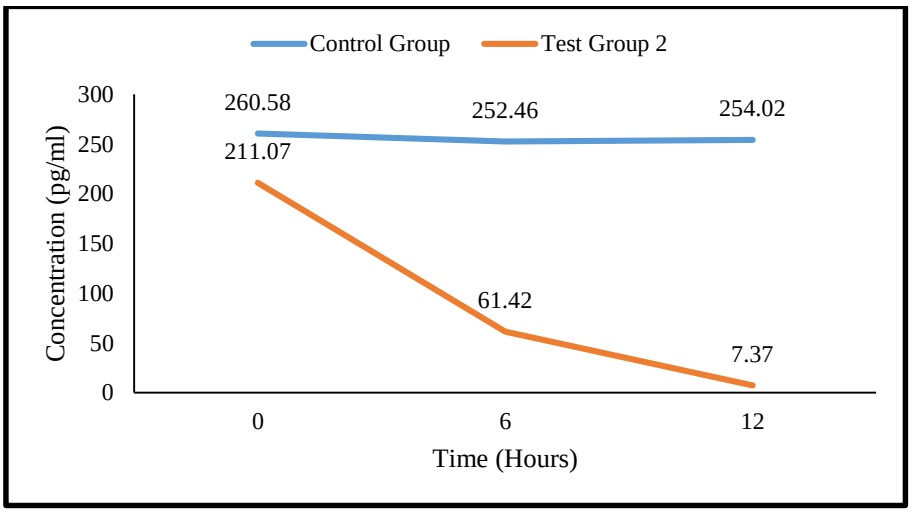


Figure 31 Timeline Comparison of mean lactate level between control and test subject group 1

5.7 INTER COMPARISON OF MOLECULAR ANALYSIS RESULTS IN CONTROL GROUP Vs TEST GROUP II

5.7.1 CK-MB LEVEL IN BLOOD

An independent-samples t-test was conducted to compare whether or not driver fatigue is associated with the increase in CK-MB level in blood plasma within the driver. For this the results of the control group was compared with the non-trained driving induced fatigued group. So after the stimulated driving session of 12hrs, the mean value of CK-MB in blood of test group-I was found to be 4.39±0.264 and to that of control group was 0.16±0.017. The results obtained from these was found to be extremely statistically significant with p value 0.181. This state that with increase in induced driving fatigue, the CK-MB level in blood of non-trained drivers with stimulated driving also increased. Though it was found that the increased CK-MB level was well within the normal range of the human but the results shows that CK-MB can be used as a biomarker for determination of progression of fatigue within an individual with prolonged work time.

5.7.2 LACTATE LEVEL IN BLOOD

An independent-samples t-test was conducted to compare whether or not driver fatigue is associated with the increase in lactate level in blood plasma within the driver. For this the results of the control group was compared with the non-trained driving induced fatigued group. So after the stimulated driving session of 12hrs, the mean value of lactate in blood of test group-I was found to be 57.29±2.49 and to that of control group was 14.15±0.370. The results obtained from these was found to be extremely statistically significant with p value 0.323. This state that with increase in induced driving fatigue, the CK-MB level in blood of non-trained drivers with stimulated driving also increased. The increased in lactate level crossed the normal value after 6hrs of driving and was recorded atleast four times that of before driving session.

5.7.3 SEROTONIN LEVEL IN BLOOD

An independent-samples t-test was conducted to compare whether or not driver fatigue is associated with the increase in serotonin level in blood plasma within the driver. So after the stimulated driving session of 12hrs, the mean value of lactate in blood of test group-I was found to be 13.90±4.65 and to that of control group was 254.02±13.691. The results obtained from these was found to be extremely statistically significant with p value 0.183. So serotonin can be used as biomarker to determine the fatigue in an individual.

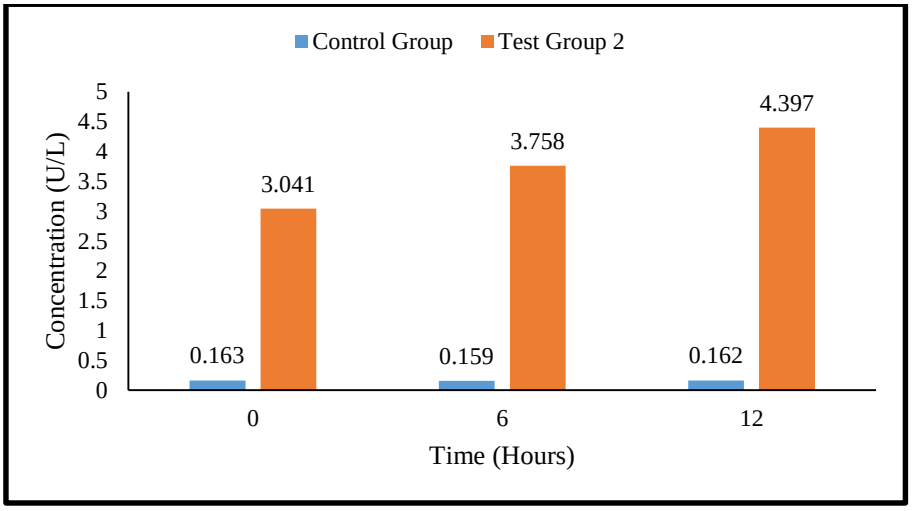


Figure 32 Bar graph Comparison of mean CK-MB level between control and test subject group 1

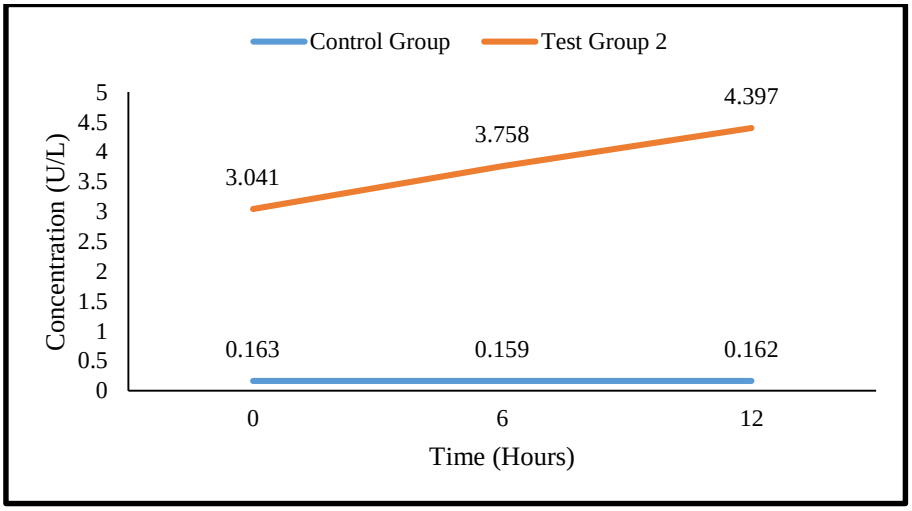


Figure 33 Timeline Comparison of mean CK-MB level between control and test subject group 1

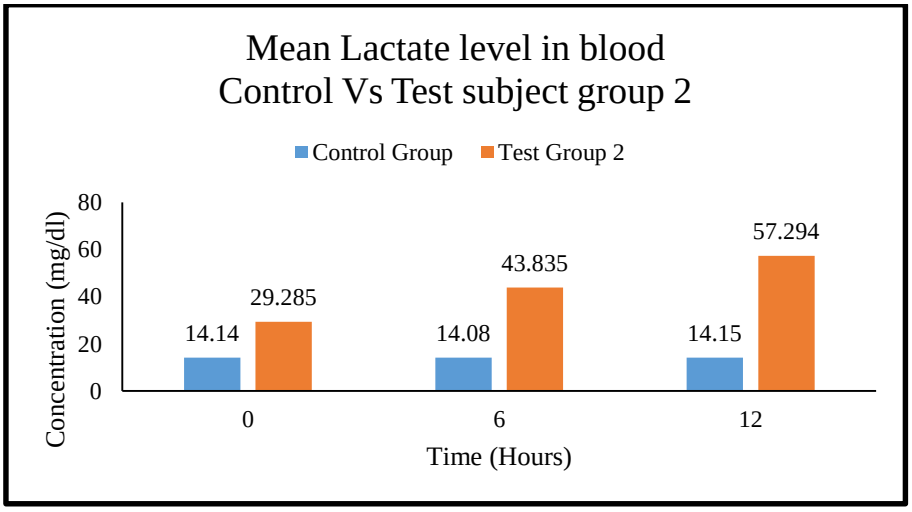


Figure 34 Bar graph Comparison of mean lactate level between control and test subject group 1

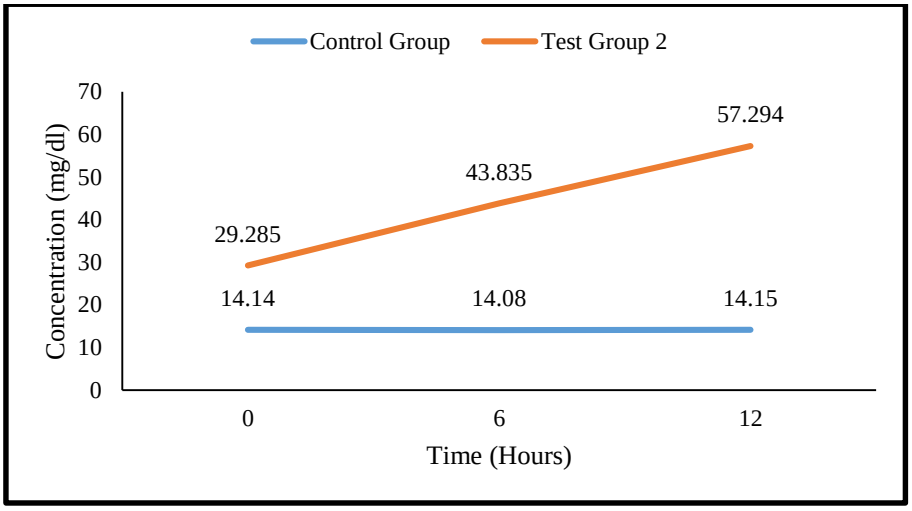


Figure 35 Timeline Comparison of mean lactate level between control and test subject group 1

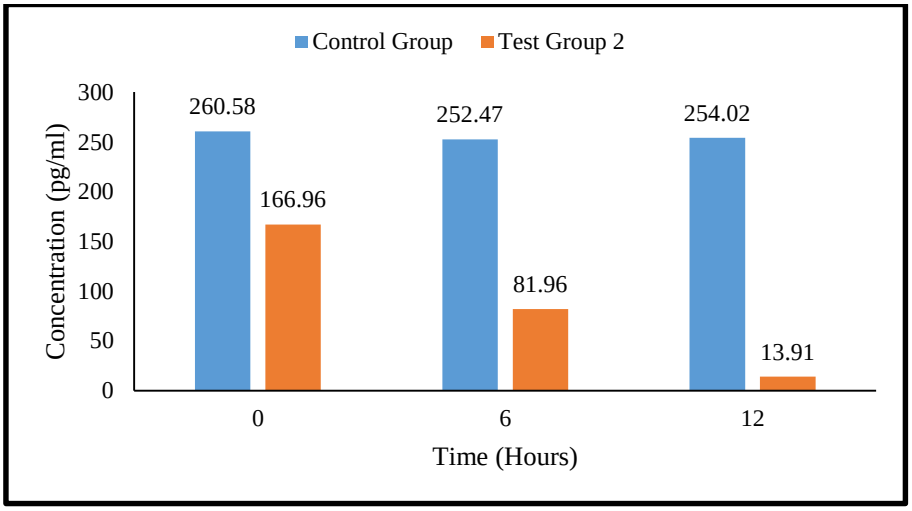


Figure 36 Bar graph Comparison of mean serotonin level between control and test subject group 1

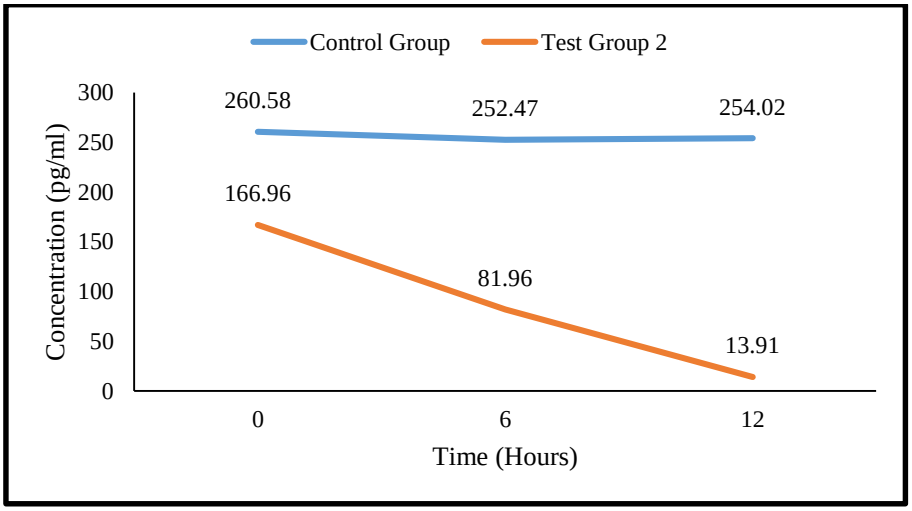


Figure 37 Timeline Comparison of mean lactate level between control and test subject group 1

5.8 INTER COMPARISON OF MOLECULAR ANALYSIS RESULTS IN TEST GROUP I Vs TEST GROUP II

5.8.1 CK-MB LEVEL IN BLOOD

An independent-samples t-test was conducted to compare whether or not driver fatigue is associated with the increase in CK-MB level in blood plasma within the driver. For this the results of the test group-I was compared with the group-II trained driving induced fatigued. So after the stimulated driving session of 12hrs, the mean value of CK-MB in blood of test group-II was found to be 4.39±0.264 and to that of test group-I was 1.70±0.055. The results obtained from these was found to be extremely statistically significant. This state that with increase in induced driving fatigue, the CK-MB level in blood of non-trained drivers with stimulated driving also increased. Though it was found that the increased CK-MB level was well within the normal range of the human but the results shows that CK-MB can be used as a biomarker for determination of progression of fatigue within an individual with prolonged work time.

5.8.2 LACTATE LEVEL IN BLOOD

An independent-samples t-test was conducted to compare whether or not driver fatigue is associated with the increase in lactate level in blood plasma within the driver. For this results of the test group-I was compared with the group-II trained driving induced fatigued. So after the stimulated driving session of 12hrs, the mean value of lactate in blood of test group-II was found to be 57.29±2.49 and to that of test group-I was 83.72±4.32. The results obtained from these was found to be extremely statistically significant with p value 0.027. This state that with increase in induced driving fatigue, the CK-MB level in blood of non-trained drivers with stimulated driving also increased. The increased in lactate level crossed the normal value after 6hrs of driving and was recorded atleast three times that of before driving session.

5.8.3 SEROTONIN LEVEL IN BLOOD

An independent-samples t-test was conducted to compare whether or not driver fatigue is associated with the increase in serotonin level in blood plasma within the driver. So after the stimulated driving session of 12hrs, the mean value of lactate in blood of test group-II was found to be 13.90±4.651 and to that of test group-I was 254.02±13.691. The results obtained from these was found to be extremely statistically significant. So serotonin can be used as biomarker to determine the fatigue in an individual.

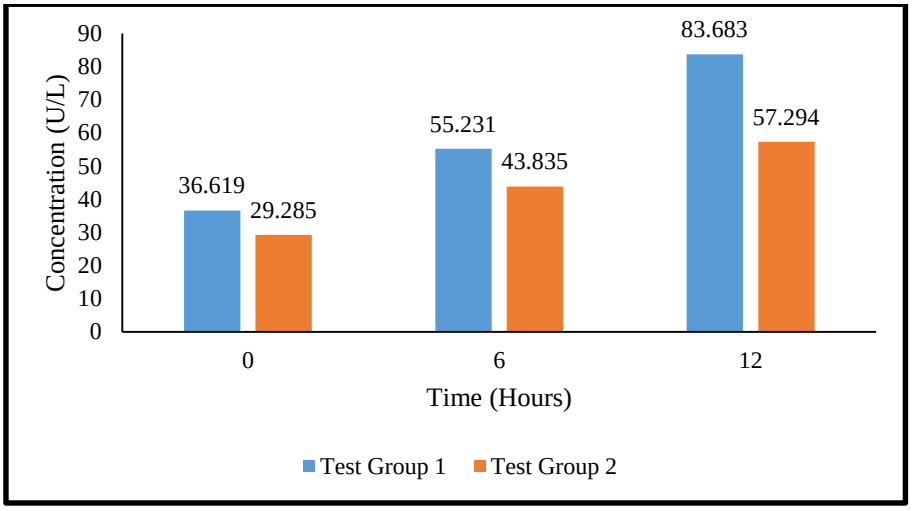


Figure 38 Bar graph Comparison of mean CK-MB level between group I and test subject group II

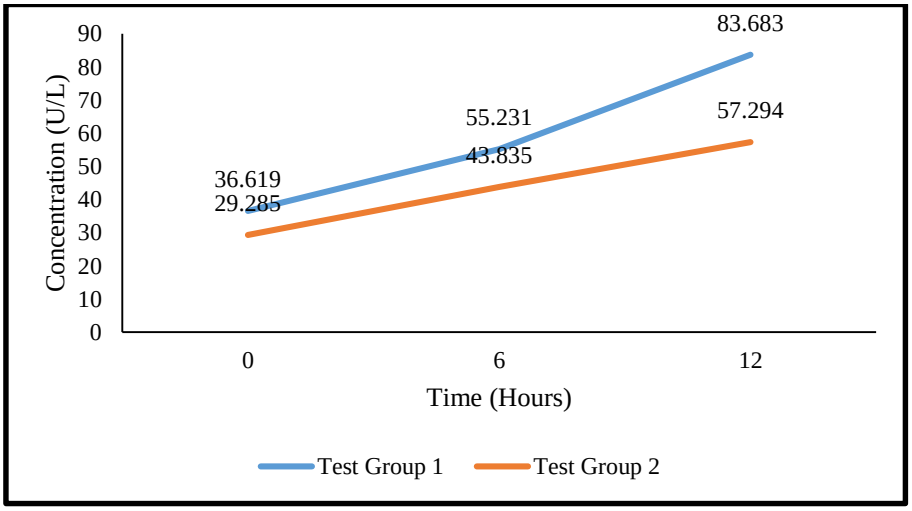


Figure 39 Timeline Comparison of mean CK-MB level between group I and test subject group II

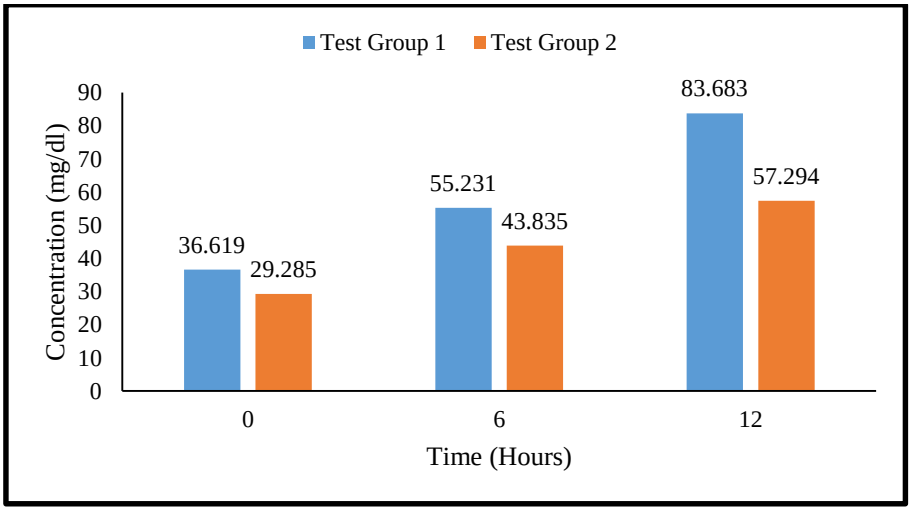


Figure 40 Bar graph Comparison of mean lactate level between group I and test subject group II

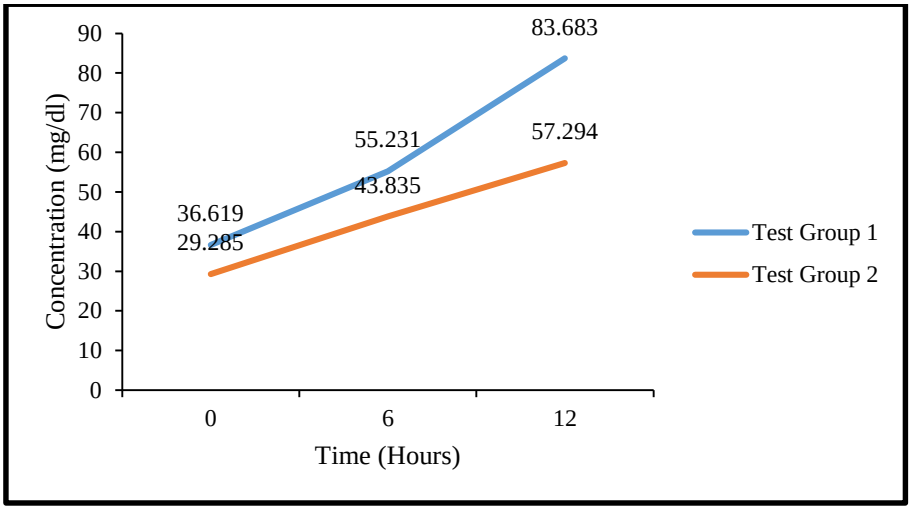


Figure 41 Timeline Comparison of mean lactate level between group I and test subject group II

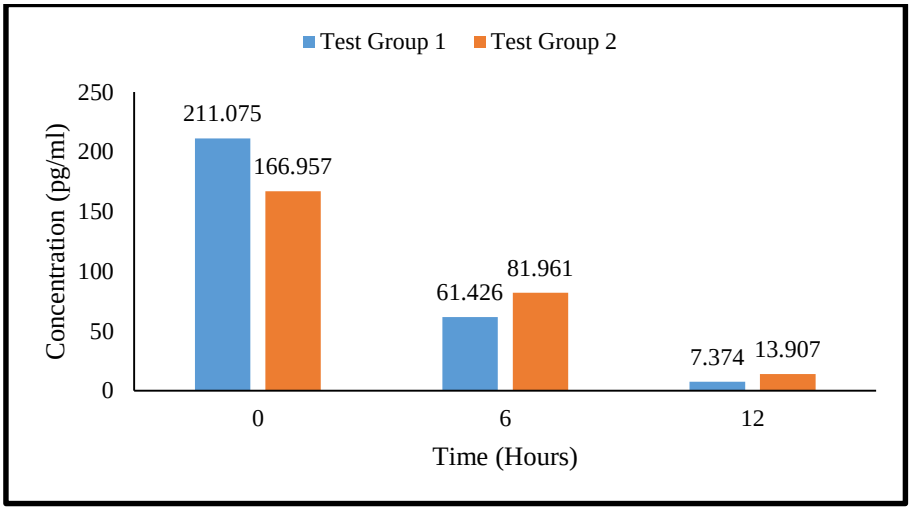


Figure 42 Bar graph Comparison of mean serotonin level between group I and test subject group II

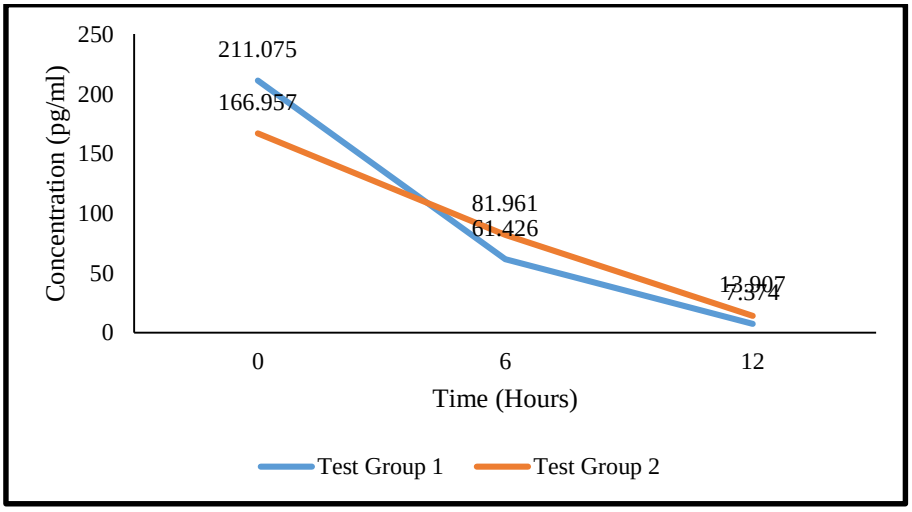


Figure 43 Timeline Comparison of mean lactate level between group I and test subject group II

6.0 CONCLUSIONS

6.1 SUMMARY:

1. The concentration of CK-MB, lactate and platelet rich plasma serotonin in blood of control group was recorded to be within the normal range and not affected during the study time of 12 hrs interval. This conclude that there was no significant effect in these biomarkers with normal day to day activities.
2. The concentration of CK-MB and lactate in blood of non-trained drivers and in trained auto-rickshaw drivers were recorded to be significantly increased with driving induced fatigue. This conclude that these blood biomarkers are related to the peripheral fatigue and can be consider for fatigue staging.
3. The concentration of platelet rich plasma in blood of non-trained drivers and in trained auto-rickshaw drivers were recorded to be significantly decreased with driving induced fatigue. This conclude that this biomarker is related to the central fatigue and can be consider to be one of the best biomarker for study for fatigue staging.
4. The subjective questionnaire based assessment used to determine the fatigue related feelings in test subjects were also proved to be among the reliable sources needed to determine fatigue in an individual.

6.2 CONCLUSION:

The final conclusion of this research is that these three biomarkers together i.e. CK-MB, lactate and platelet rich plasma serotonin can be used as a reliable markers for fatigue staging in drivers.

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APPENDIX I

LABORATORY STUDY INFORMED CONSENT FORM

NATIONAL INSTITUTE OF TECHNOLOGY ROURKELA

Informed Consent for Participants
In Research Projects Involving Human Subjects

Title of Project: Estimation of the driver fatigue based on the simulating driving session.

Investigator(s): Rakesh Buhlan (Mtech), Ritu Mishra (Btech), Dr B P Nayak (Faculty Advisor)

1) Purpose

The purpose of this project is to examine fatigue and performance during simulated driving work task. Specifically, we are interested in determining how long hours of driving affects the performance of an individual (normal drivers/professional drivers) on cellular, mental and physical level. The results of the study will help us to understand how fatigue can impact performance and safety of an individual.

2) Procedures

It is important for you to understand that we are not evaluating you or your abilities in any way. You are helping us to collect data that will be used to understand how work demands affect Fatigue and performance in driving tasks. Therefore, we ask that you perform normally and be as honest as possible in your responses to questions. The information and feedback that you provide is very important to this project. The experiment will consist simulating driving session of 12 hours. During this time, you will be asked to give the blood samples three times i.e. before, middle and at the end of the driving in order to determine the level of specific biomarkers i.e. serotonin, ck-mb and lactate within the blood.

During the course of this experiment you will be asked to perform the following tasks:

1) Read and sign an Informed Consent Form (this form)
2) Fill out a brief demographic form
3) Perform simulated driving for 12 hours.
4) Fill the subjective questionnaire i.e. Epworth Sleepiness Scale (ESS), Berlin Questionnaire (BQ), Pittsburgh
Sleep Quality Index (PSQI) and the Beck Depression Inventory (BDI).

If you agree to be in this study, you will go to *cell and molecular biology lab-II, department of Biotechnology and Medical Engineering, NIT Rourkela* and give a blood sample. The blood will be drawn by putting a needle into a vein in your arm. One small tube of blood will be taken. This will take about five minutes. *The blood (3-4ml) will be drawn three time during the session i.e. before, at 6 hours and after the simulated driving.*

3) Risks and Benefits

There are minimal risks to you as a participant in this study as follows.

1. You may experience minor muscle strain or fatigue as a result of performing the experimental tasks.
2. You may experience some muscle soreness, 1-2 days after the experiment.
3. The needle stick may hurt. There is a small risk of bruising and fainting, and a rare risk of infection.

Participants in a study are considered volunteers, regardless of whether they receive payment for their participation. Should you be injured during your participation in this study, neither the researchers or the Institute have money set aside to pay for medical treatment, and any costs associated with treatment would be at your own expense.
This research project will help quantify the effects of fatigue on performance. While this research may yield such benefits, no promise or guarantee of benefits will be made to participants. Participants may contact the investigators listed at the end of the Consent Form to inquire about the results and conclusions of this research.

4) Extent of Anonymity and Confidentiality

Your personal information and identity will be kept in the strictest of confidence. No names will appear on questionnaires or surveys, and a coding system will be used to associate your identity with questionnaire answers and data. The list associating names with answers will be destroyed one month after completion of data collection. All information will be collected in a file and locked when not being used, and only the investigators have access to the data.

5) Informed Consent

You will receive two informed consent forms to be signed before beginning the experiment; one for your record and one for the experimenter's record.

6) Compensation

If you meet all of the inclusion criteria, there will be no compensation awarded for participating in this research work.

7) Freedom to Withdraw

You are free to withdraw from this study at any time without penalty or reason stated, and no penalty or withholding of compensation will occur for doing so. Furthermore, you are free not to answer any question or respond to experimental situations without penalty. There may be circumstances under which the investigator may determine that the experiment should not be continued. In this case, you will be compensated for the portion of the project completed.

8) Participant's Responsibilities

I voluntarily agree to participate in this study. I have the following responsibilities:

1. To read and understand all instructions.
2. To work under the conditions specified by the experimenter to the best of my ability.
3. To answer questions, surveys, etc. honestly and to the best of my ability.
4. To inform the investigator of any discomforts I experience immediately.
5. Be aware that I am free to ask questions at any point.
6. Be aware that I am free to ask questions at any point.

9) Participant's Permission

I have read the Consent Form and conditions of this project and the answers I have included within the declaration of physical health are factual. I have had all my questions answered. I hereby acknowledge the above and give my voluntary consent to participate with the understanding that I may discontinue participation at any time if I choose to do so:

Date Participant's Signature

Date Experimenter's

Signature

The research team for this experiment is led by Dr. Bibhukalyan Prasad Nayak. He may be contacted at the following address and phone number:

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APPENDIX II

1 DEMOGRAPHICS QSTS

Q1) What is your age group? (please tick)	
1) Below 21 years	
2) 21 - 25	
3) 26 - 30	
4) 31 - 35	
5) 36 - 40	
6) 41 – 45	
7) Above 45	

Q2) What is your gender? (please tick)	
1) Male	
2) Female	

Q3) How long have you been employed as an auto rickshaw drive worker?	
1) Below 1 year	
2) 1 – 5 years	
3) 5 – 10 years	
4) 10 – 15 years	
5) Above 15 years	

Q4) Have you experienced fatigue in the last 6 months?	
1) Yes	
2) No	

5. If so, what do you believe this is a result of?

Q6) Do you believe fatigue has affected your performance at work?	
1) Yes	
2) No	

Q7) Have you ever been diagnosed with narcolepsy, restless leg syndrome, obstructive or central sleep apnoea?	
1) Yes	
2) No	

Q8) If so are you being treated for this?	
1) Yes	
2) No	

2 EPWORTH SLEEPINESS SCALE (JOHNS, 1991)

How likely are you to doze off or fall asleep in the following situations (rather than just feeling tired) - referring to your usual way of life in recent times. If you haven't done some of these things recently, try and work out how it would have affected you - so all questions have an answer.

Use the following scale to choose the most appropriate number for each situation
0 = no chance of dozing
1 = slight chance of dozing
2 = moderate chance of dozing
3 = high chance of dozing

SITUATION CHANCE OF DOZING

1. Sitting and reading _____
2. Watching television _____
3. Sitting, inactive in a public place (eg. in cinema or a meeting) _____
4. Passenger in a car for an hour without a break _____
5. Lying down to rest in the afternoon (when circumstances permit) _____
6. Sitting and talking to someone _____
7. Sitting quietly after lunch (without alcohol) _____

3 BERLIN QUESTIONNAIRE (FOR SLEEP APNOEA)

Q1) Do you snore?	
6) Yes	
7) No	
8) Don't Know	

If you snore:

Q2) Your snoring is	
1) Slightly louder than your breathing	
2) Louder than talking	
3) As loud as talking	
4) Very Loud	

Q3) How often do you snore?	
1) Almost every day	
2) 3-4 times a week	
3) 1-2 times a week	
4) Never or almost never	

Q4) Does your snoring bother other people?	
8) Yes	
9) No	

Q5) Has anyone ever noticed that you quit breathing during your sleep?	
1) Nearly every day	
2) 3-4 times a week	
3) 1-2 times a week	

4) 1-2 times a month	
5) Never or nearly never	
Q6) How often do you feel tired or fatigued after you sleep?	
1) Nearly every day	
2) 3-4 times a week	
3) 1-2 times a week	
4) 1-2 times a month	
5) Never or nearly never	

Q7) During your wake time, do you feel tired, fatigued or not up to par?	
1) Nearly every day	
2) 3-4 times a week	
3) 1-2 times a week	
4) 1-2 times a month	
5) Never or nearly never	

Q8) Have you ever nodded off or fallen asleep while driving a vehicle?	
1) Yes	
2) No	

If Yes,

Q9) How often does this occur?	
1) Nearly every day	
2) 3-4 times a week	
3) 1-2 times a week	
4) 1-2 times a month	
5) Never or nearly never	

Q10) **Do you have high blood pressure?**	
1) Yes	
2) No	
3) Don’t Know	

4 BECK'S DEPRESSION INVENTORY

Q1) Sadness	
1) I do not feel sad.	
2) I feel sad	
3) I am sad all the time and I can't snap out of it.	
4) I am so sad and unhappy that I can't stand it.	

Q2) Pessimism	
1) I am not particularly discouraged about the future.	
2) I feel discouraged about the future.	
3) I feel I have nothing to look forward to.	
4) I feel the future is hopeless and that things cannot improve.	

Q3) Past Failure	
1) I do not feel like a failure.	
2) I feel I have failed more than the average person.	
3) As I look back on my life, all I can see is a lot of failures.	
4) I feel I am a complete failure as a person.	

Q4) Loss of Pleasure	
1) I get as much satisfaction out of things as I used to.	
2) I don't enjoy things the way I used to.	
3) I don't get real satisfaction out of anything anymore.	
4) I am dissatisfied or bored with everything.	

Q5) Guilty Feelings	
1) I don't feel particularly guilty.	
2) I feel guilty a good part of the time.	
3) I feel quite guilty most of the time.	
4) I feel guilty all of the time.	

Q6) Punishment Feelings	
1) I don't feel I am being punished.	
2) I feel I may be punished.	
3) I expect to be punished.	
4) I feel I am being punished.	

Q7) Self Dislike	
1) I don't feel disappointed in myself.	
2) I am disappointed in myself.	
3) I am disgusted with myself.	
4) I hate myself.	

Q8) Self Criticalness	
1) I don't feel I am any worse than anybody else.	
2) I am critical of myself for my weaknesses or mistakes.	
3) I blame myself all the time for my faults.	
4) I blame myself for everything bad that happens.	

Q9) Suicidal thoughts or wishes	
1) I don't have any thoughts of killing myself.	
2) I have thoughts of killing myself, but I would not carry them out.	
3) I would like to kill myself.	

4) I would kill myself if I had the chance.	

Q10) Crying	
1) I don't cry any more than usual.	
2) I cry more now than I used to.	
3) I cry all the time now.	
4) I used to be able to cry, but now I can't cry even though I want to.	

Q11) Agitation	
1) I am no more irritated by things than I ever was.	
2) I am slightly more irritated now than usual.	
3) I am quite annoyed or irritated a good deal of the time.	
4) I feel irritated all the time.	

Q12) Loss of Interest	
1) I have not lost interest in other people.	
2) I am less interested in other people than I used to be.	
3) I have lost most of my interest in other people.	
4) I have lost all of my interest in other people.	

Q13) Indecisiveness	
1) I make decisions about as well as I ever could.	
2) I put off making decisions more than I used to.	
3) I have greater difficulty in making decisions more than I used to.	
4) I can't make decisions at all anymore.	

Q14) Worthlessness	
1) I don't feel that I look any worse than I used to.	
2) I am worried that I am looking old or unattractive.	
3) I feel permanently unattractive.	
1) I believe that I look ugly.	

Q15) Loss of Energy	
1) I can work about as well as before.	
2) It takes an extra effort to get started at doing something.	
3) I have to push myself very hard to do anything.	
4) I can't do any work at all.	

Q16) Changes in sleeping pattern	
1) I can sleep as well as usual.	
2) I don't sleep as well as I used to.	
3) I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.	
4) I wake up several hours earlier than I used to and cannot get back to sleep.	
Q17) Irritability	
1) I don't get more tired than usual.	
2) I get tired more easily than I used to.	
3) I get tired from doing almost anything.	
4) I am too tired to do anything.	

Q18) Changes in appetite	
1) My appetite is no worse than usual.	
2) My appetite is not as good as it used to be.	
3) My appetite is much worse now.	

4) I have no appetite at all anymore.	

Q19) Concentration difficulty	
1) I haven't lost much weight, if any, lately.	
2) I have lost more than five pounds.	
3) I have lost more than ten pounds.	
4) I have lost more than fifteen pounds.	

Q20) Tiredness or Fatigue	
1) I am no more worried about my health than usual.	
2) I am worried about physical problems	
3) I am very worried about physical problems and it's hard to think of much else.	
4) I am so worried about my physical problems that I cannot think of anything else.	